Metabolic impact of pheochromocytoma/paraganglioma: Targeted metabolomics in patients before and after tumor removal

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Abstract: Objective: Excess catecholamine release by pheochromocytomas and paragangliomas (PPGL) leads to characteristic clinical features and increased morbidity and mortality. The influence of PPGLs on metabolism is ill described but may impact diagnosis and management. The objective of this study was to systematically and quantitatively study PPGL induced metabolic changes at a systems level. Design: Targeted metabolomics by liquid chromatography-tandem mass spectrometry of plasma specimens in a clinically well characterized prospective cohort study. Methods: Analyses of metabolic profiles of plasma specimens from 56 prospectively enrolled and clinically well characterized patients (23 males, 33 females) with catecholamine-producing PPGL before and after surgery, as well as measurement of 24h-urinary catecholamine using LC-MS/MS. Results: From 127 analyzed metabolites, 15 were identified with significant changes before and after surgery: 5 amino acids/biogenic amines (creatinine, histidine, ornithine, sarcosine, tyrosine) and 1 glycerophospholipid (PCaeC34:2) with increased concentrations and 6 glycerophospholipids (PCaaC38:1, PCaaC42:0, PCaeC40:2, PCaeC42:5, PCaeC44:5, PCaeC44:6), 2 sphingomyelins (SMC24:1, SMC26:1) and hexose with decreased levels after surgery. Patients with a noradrenergic tumor phenotype had more pronounced alterations compared to those with an adrenergic tumor phenotype. Weak, but significant correlations for 8 of these 15 metabolites with total urine catecholamine levels were identified. Conclusions: This first large prospective metabolomics analysis of PPGL patients demonstrates broad metabolic consequences of catecholamine excess. Robust impact on lipid and amino acid metabolism may contribute to increased morbidity of PPGL patients.

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Supplemental Material

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Table 1a. List of metabolites measured with the AbsoluteIDQ® p180 Kit included in statistical analyses (127 of 188)**

Acylcarnitines§	*Amino acids and biogenic amines (cont.)*	*Glycero-phospholipids (cont.)*	*Glycero-phospholipids (cont.)*	*Sphingolipids*
C0 (Carnitine)	SDMA (Symmetric diemthylarginine)	PC aa C36:4	PC ae C36:0*	SM (OH) C14:1
C2 (Acetylcarnitine)	Sarcosine*	PC aa C36:5	PC ae C36:1	SM (OH) C16:1
C14:1 (Tetradecanoylcarnitine)*	Taurine	PC aa C36:6	PC ae C36:2	SM (OH) C22:1
C18:1 (Octadecenoylcarnitine)	***Glycerophospholipids***	PC aa C38:0	PC ae C36:3	SM (OH) C22:2
C18:2 (Octadecadienylcarnitine)	lysoPC a C16:0	PC aa C38:1	PC ae C36:4	SM (OH) C24:1
Amino acids and biogenic amines	lysoPC a C16:1	PC aa C38:3	PC ae C36:5	SM C16:0
Ala (Alanine)	lysoPC a C17:0	PC aa C38:4	PC ae C38:0	SM C16:1
Arg (Arginine)	lysoPC a C18:0	PC aa C38:5	PC ae C38:1*	SM C18:0
Asn (Asparagine)	lysoPC a C18:1	PC aa C38:6	PC ae C38:2	SM C18:1
Cit (Citrulline)	lysoPC a C18:2	PC aa C40:2	PC ae C38:3	SM C20:2
Gln (Glutamine)	lysoPC a C20:3	PC aa C40:3	PC ae C38:4	SM C24:0
Glu (Glutamate)*	lysoPC a C20:4	PC aa C40:4	PC ae C38:5	SM C24:1
Gly (Glycine)	lysoPC a C24:0	PC aa C40:5	PC ae C38:6	SM C26:0*
His (Histidine)	lyso PC a C26:0*	PC aa C40:6	PC ae C40:1	SM C26:1
Ile (Isoleucine)	lysoPC a C28:1	PC aa C42:0	PC ae C40:2	**Monosaccharides**
Leu (Leucine)	PC aa C28:1	PC aa C42:1	PC ae C40:3	H1 (sum of hexoses)
Lys (Lysine)	PC aa C30:0	PC aa C42:2*	PC ae C40:4	
Met (Methionine)	PC aa C32:0	PC aa C42:4	PC ae C40:5	
Orn (Ornithine)	PC aa C32:1	PC aa C42:5	PC ae C40:6	
Phe (Phenylalanine)	PC aa C32:2	PC aa C42:6	PC ae C42:1*	
Pro (Proline)	PC aa C32:3	PC ae C30:0	PC ae C42:2	
Ser (Serine)	PC aa C34:1	PC ae C30:1*	PC ae C42:3	
Thr (Threonine)	PC aa C34:2	PC ae C30:2	PC ae C42:4*	
Trp (Tryptophan)	PC aa C34:3	PC ae C32:1	PC ae C42:5	
Tyr (Tyrosine)	PC aa C34:4	PC ae C32:2	PC ae C44:4	
Val (Valine)	PC aa C36:0	PC ae C34:0	PC ae C44:5	
ADMA (Asymmetric dimethylarginine)*	PC aa C36:1	PC ae C34:1	PC ae C44:6	
Creatinine	PC aa C36:2	PC ae C34:2		
Kynurenine	PC aa C36:3	PC ae C34:3		

* metabolites with missing values (less than 10%), §Cx:y indicates the lipid chain composition where “x” is the number of carbons and “y” the number of double bonds

** 127 of 183 metabolites were included in the analysis of all PPGL patients for before-after surgery comparison; 126 of 183 metabolites were considered for before-after surgery comparison of the male patients, patients with BMI ≥ 25 kg/m2 and with age <45 years (Sarcosine additionally missing in >10%) as well as patients with noradrenergic tumor phenotype (PC ae C30:1 additionally missing in >10%); 125 of 183 metabolites were considered for before-after surgery comparison of patients with adrenergic tumor phenotype (C14:1 and Sarcosine additionally missing in >10%) and BMI <25 kg/m2 (C14:1 and PC ae C30:1 additionally missing in >10%); 124 of 183 were considered for preoperative comparison of patients according to sex, age, tumor localization, catecholamine phenotype, presence/absence of arterial hypertension and diabetes mellitus (C14:1, Sarcosine and PC ae 30:1 additionally missing >10%).

Abbreviations: lysoPC, lysophosphatidylcholine, PC, phosphatidylcholine; a, acyl; aa, diacyl; ae, acyl-alkyl; PPGL, pheochromocytoma/paraganglioma; SM, sphingomyelin; SM(OH), hydroxysphingomyelin

Table 1b: List of metabolites measured with the Absolute*IDQ*® p180 Kit excluded from the statistical analyses (61 of 188)

Acylcarnitines	**Acylcarnitines (cont.)**	**Amino acids and biogenic amines (cont.)**
C3 (Propionylcarnitine)	C14:1-OH (Hydroxytetradecenoylcarnitine)	Putrescine
C3:1 (Propenoylcarnitine)	C14:2 (Tetradecadienylcarnitine)	Serotonin
C3-OH (Hydroxypropionylcarnitine)	C14:2-OH (Hydroxytetradecadienylcarnitine)	Spermidine
C4 (Butyrylcarnitine)	C16 (Hexadecanoylcarnitine)	Spermine
C4:1 (Butenoylcarnitine)	C16:1 (Hexadecenoylcarnitine)	**Glycerophospholipids**
C4-OH (C3-DC) (Hydroxybutyrylcarnitine)	C16:1-OH (Hydroxyhexadecenoylcarnitine)	lysoPC a C14:0
C5 (Valerylcarnitine)	C16:2 (Hexadecadienylcarnitine)	lysoPC a C26:1
C5:1 (Tiglylcarnitine)	C16:2-OH (Hydroxyhexadecadienylcarnitine)	lysoPC a C28:0
C5:1-DC (Glutaconylcarnitine)	C16-OH (Hydroxyhexadecanoylcarnitine)	PC aa C24:0
C5-DC (C6-OH) (Glutarylcarnitine/Hydroxyhexanoylcarnitine)	C18 (Octadecanoylcarnitine)	PC aa C26:0
C5-M-DC (Methylglutarylcarnitine)	C18:1-OH (Hydroxyoctadecenoylcarnitine)	PC aa C30:2
C5-OH (C3-DC-M) (Hydroxyvalerylcarnitine/Methylmalonylcarnitine)	**Amino acids and biogenic amines**	PC aa C40:1
C6 (C4:1-DC) (Hexanoylcarnitine/Fumarylcarnitine)	Asp (Aspartate)	PC ae C42:0
C6:1 (Hexenoylcarnitine)	Ac-Orn (Acetylornithine)	PC ae C44:3
C7-DC (Pimelylcarnitine)	alpha-AAA (alpha-Aminoadipic acid)	**Sphingolipids**
C8 (Octanoylcarnitine)	Carnosine	SM C22:3
C9 (Nonanoylcarnitine)	DOPA	
C10 (Decanoylcarnitine)	Dopamine	
C10:1 (Decenoylcarnitine)	Histamine	
C10:2 (Decadienylcarnitine)	Met-SO (Methionine sulfoxide)	
C12 (Dodecanoylcarnitine)	Nitro-Tyr (Nitrotyrosine)	
C12:1 (Dodecenoylcarnitine)	PEA (Phenylethylamine)	
C12-DC (Dodecanedioylcarnitine)	*cis*-OH-Pro (*cis*-4-Hydroxyproline)	
C14 (Tetradecanoylcarnitine)	*trans*-OH-Pro (*trans*-4-Hydroxyproline)	

* metabolites with missing values (less than 10%), §Cx:y indicates the lipid chain composition where “x” is the number of carbons and “y” the number of double bonds

Abbreviations: lysoPC, lysophosphatidylcholine, PC, phosphatidylcholine; a, acyl; aa, diacyl; ae, acyl-alkyl; PPGL, pheochromocytoma/paraganglioma; SM, sphingomyelin; SM(OH), hydroxysphingomyelin

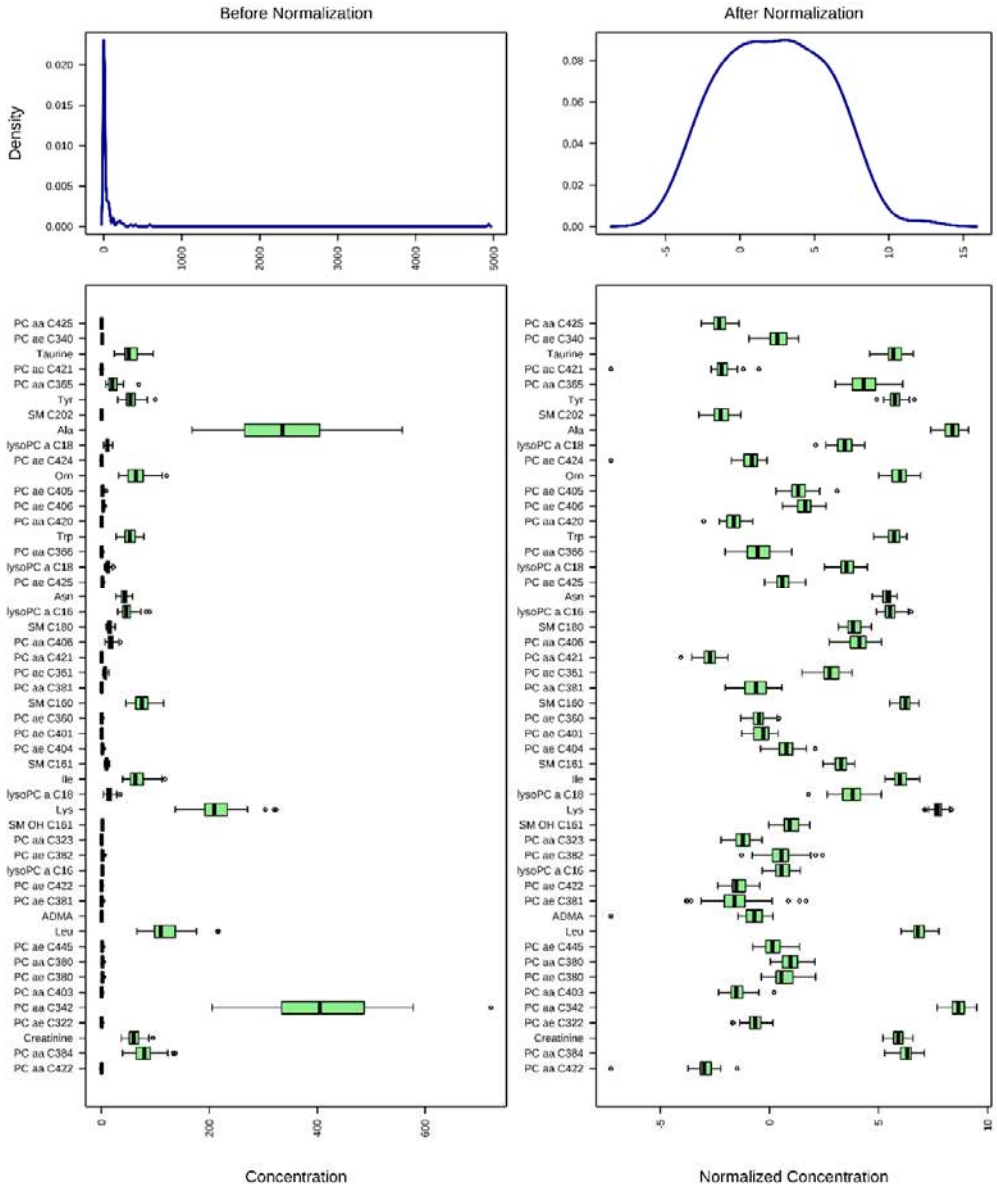
2. Statistical analyses results:

2.1 Targeted metabolomics prior surgery: metabolic pattern differences according to sex (a), tumor localization (b), presence of metastases (c), catecholamine phenotype (d), BMI (e), presence of arterial hypertension (f) and diabetes mellitus (g).

2.1 a) Statistical analyses for sex comparison (female/male) in PPGL patients prior surgery.

- Normalization Result (all 56 preoperative patients/samples):

The boxplots (below) show at most 50 features due to space limitation; the density plots (above) are based on all data.



- Results of the performed statistical tests:

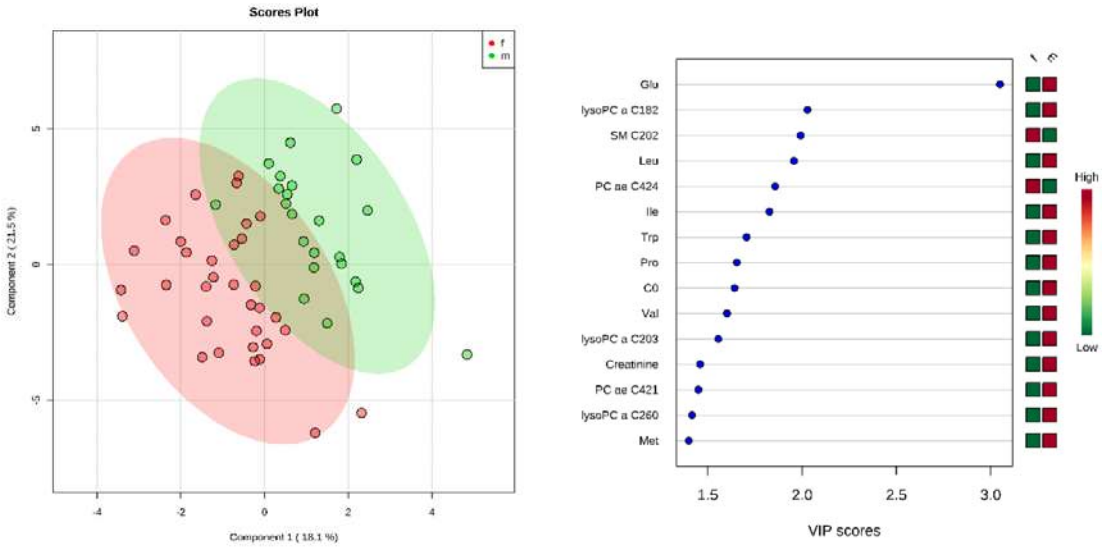
***Significant metabolites identifed by* t-test analysis**						***Significant metabolites identified by fold change analysis***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Fold**	**log2(FC)**
Leu	-6.1393	1.02E-07	6.9919	1.26E-05		Ala	0.83316	-0.26334
Ile	-5.3479	1.85E-06	5.7333	0.00011457		C0	0.78251	-0.35383
Val	-5.2337	2.79E-06	5.555	0.00011517		Creatinine	0.80142	-0.31937
Trp	-4.9884	6.68E-06	5.1753	0.00020706		Glu	0.67663	-0.56357
Creatinine	-4.3717	5.65E-05	4.2476	0.0010016		His	0.82306	-0.28094
Met	-4.4024	5.10E-05	4.2927	0.0010016		Ile	0.74515	-0.4244
SM C202	4.4357	4.55E-05	4.3418	0.0010016		Leu	0.72958	-0.45487
C0	-4.324	6.64E-05	4.1778	0.0010293		lysoPC a C181	0.82957	-0.26957
His	-4.0766	0.00015111	3.8207	0.0020819		lysoPC a C182	0.76175	-0.3926
Phe	-4.0093	0.00018829	3.7252	0.0023348		lysoPC a C203	0.77832	-0.36156
Tyr	-3.6378	0.00061534	3.2109	0.0069365		lysoPC a C204	0.80518	-0.31262
Pro	-3.551	0.00080479	3.0943	0.0083162		Met	0.80187	-0.31856
Glu	-3.3356	0.0015445	2.8112	0.014732		PC aa C323	1.2576	0.33069
SM C181	3.2224	0.0021564	2.6663	0.0191		PC aa C343	1.2177	0.28419
lysoPC a C203	-2.928	0.0049863	2.3022	0.04122		PC ae C403	1.2233	0.29078
Significant metabolites identified by EBAM						SM C181	1.1978	0.2604
Name	**z.value**	**posterior**	**local.fdr**			SM C181	1.2216	0.28877
Leu	6.1393	0.99994	6.15E-05			SM C202	1.3779	0.46244
Ile	5.3479	0.99953	0.00046729			Trp	0.77625	-0.3654
Val	5.2337	0.99937	0.0006251			Tyr	0.81918	-0.28775
Trp	4.9884	0.99883	0.0011656			Val	0.77765	-0.3628
Met	4.4024	0.99493	0.0050749					
Creatinine	4.3717	0.99452	0.0054771					
C0	4.324	0.99384	0.0061649					
His	4.0766	0.98866	0.011341					
Phe	4.0093	0.98663	0.01337					
SM C202	-4.4357	0.97999	0.020011					
Tyr	3.6378	0.96715	0.032848					
Pro	3.551	0.95959	0.040411					
Glu	3.3356	0.93273	0.067271					
Significant metabolites identified by SAM								
Name	**d.value**	**stdev**	**rawp**	**q.value**				
Leu	2.1722	0.072345	0	0				
Glu	2.0446	0.20926	8.06E-05	0.0021306				
Ile	1.9996	0.078905	8.06E-05	0.0021306				
SM C202	-1.9648	0.10507	0.00016129	0.0031959				
Trp	1.8353	0.076904	0.00040323	0.0063918				
Val	1.8084	0.069754	0.00064516	0.0085224				
C0	1.715	0.086857	0.0010484	0.01187				
Pro	1.6047	0.10894	0.0024194	0.023969				
Creatinine	1.5588	0.073222	0.0031452	0.024928				
Met	1.5737	0.073504	0.0029032	0.024928				
lysoPC a C182	1.516	0.16088	0.0039516	0.028473				
His	1.4353	0.071796	0.0057258	0.037818				
Tyr	1.39	0.081705	0.0074194	0.045234				
lysoPC a C203	1.3625	0.11499	0.0087903	0.049765				

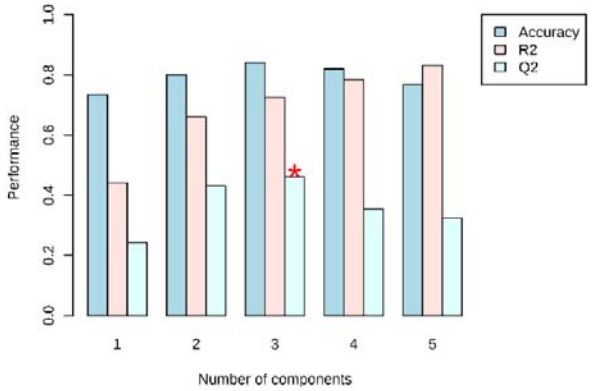
For the fold change analysis the fold change threshold was set at 1.2. For SAM analysis the delta value was at 0.7 for an FDR value of 0.032; the q.value represent the FDR-adjusted p.value. For EBAM the delta value was set at 0.9 with an FDR of 0.025. **Abbreviations:** FDR, false discovery rate;

n.s., not significant; SAM, significance analysis of microarray/metabolites/metabolite; EBAM, empirical bayesian analysis of microarray/metabolites/metabolite.

- PLS-DA results:

In the left upper corner the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the upper right corner are represented the important features of the component with higher percentage of explained variability (in this case component 2). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study ("f" for female and "m" for male). In the lower image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2 (images and commentary from the Metaboanalyst (1)).

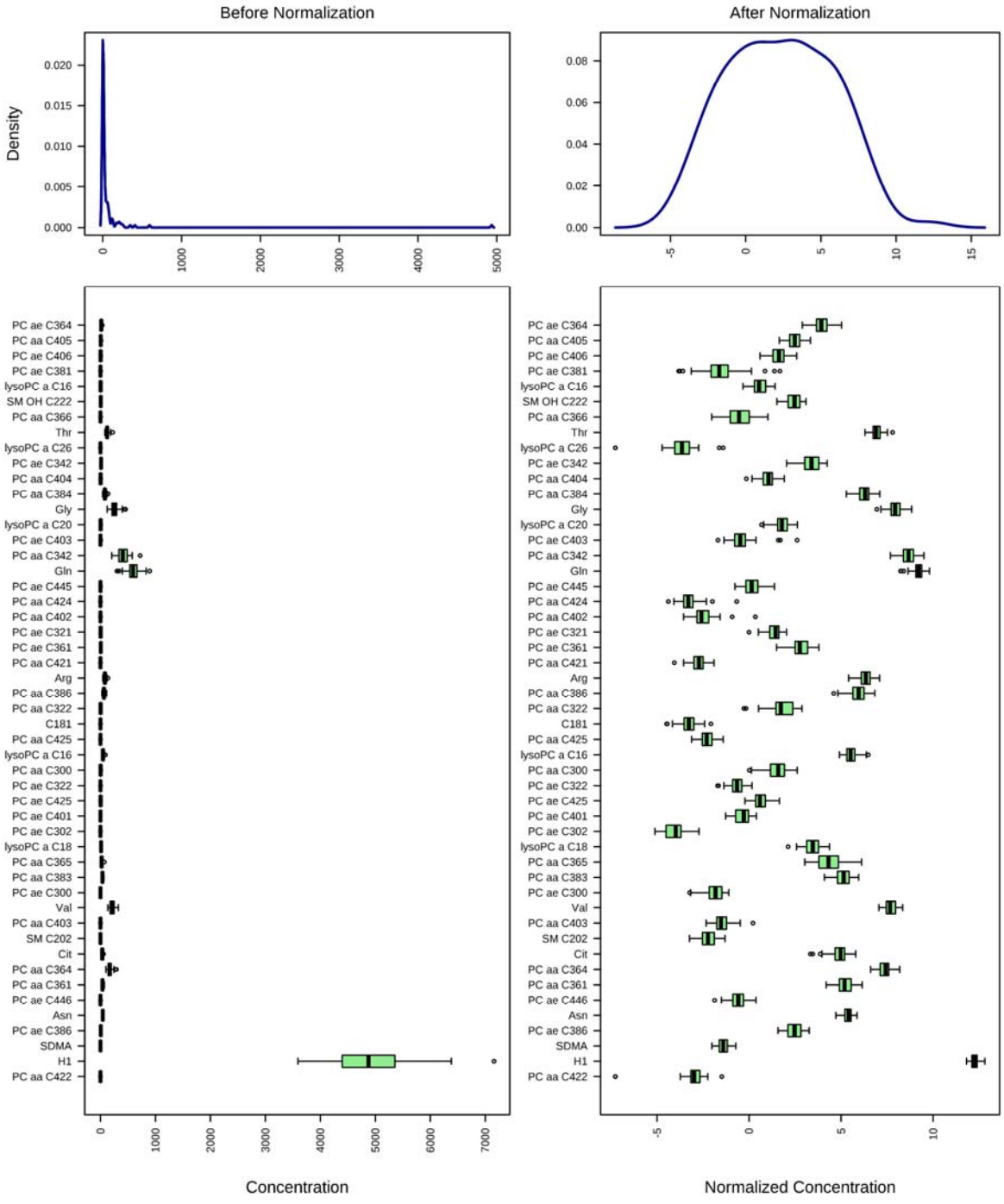




2.1 b) Statistical analyses for tumor localization (adrenal/extraadrenal) in PPGL patients prior surgery

- Normalization Result (56 preoperative patients/samples):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



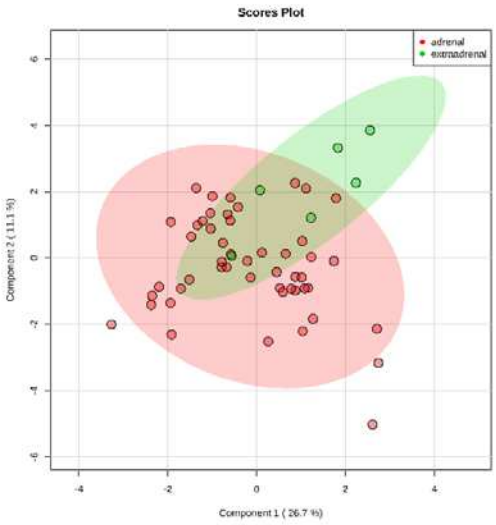
- Results of the performed statistical tests:

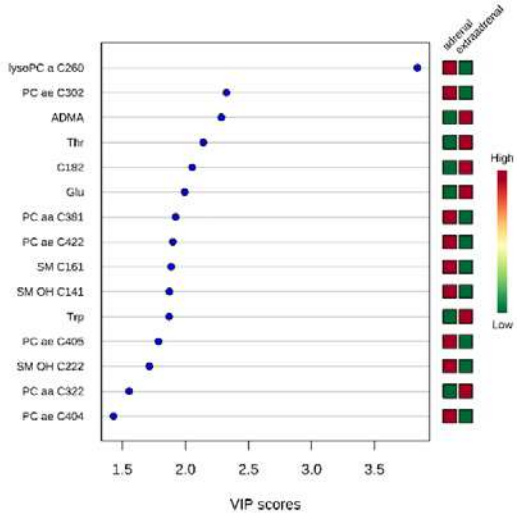
***Metabolites (first 10) identifed by* t-test analysis (none resulted significant after correction for FDR)**						***Metabolites identified by fold change analysis (adrenal VS extraadrenal)***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Fold Change**	**log2(FC)**
Thr	-2.1054	0.039922	1.3988	0.99572		PC ae C381	1.413	0.49875
Trp	-1.5944	0.11669	0.93298	0.99572		PC ae C403	1.2811	0.3574
SM C161	1.5257	0.13293	0.87639	0.99572		PC ae C302	1.2415	0.31211
PC ae C422	1.364	0.17822	0.74905	0.99572		PC ae C405	1.2311	0.29993
PC ae C302	1.318	0.19307	0.71429	0.99572		PC aa C381	1.2205	0.28749
His	-1.2572	0.21409	0.6694	0.99572		PC ae C422	1.2151	0.28103
SM OH C222	1.2055	0.23328	0.63212	0.99572		Thr	0.82619	-0.27545
C182	-1.1881	0.24001	0.61978	0.99572		PC ae C382	1.2065	0.27082
lysoPC a C260	1.183	0.242	0.61619	0.99572				
Met	-1.1397	0.25944	0.58597	0.99572				
Metabolites identified by EBAM								
none								
Metabolites identified by SAM								
none								

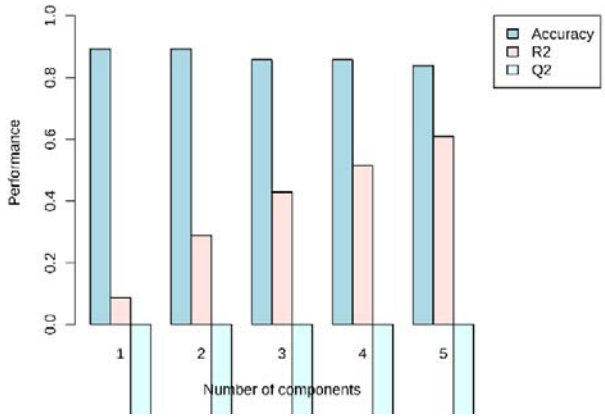
For the fold change analysis the fold change threshold was set at 1.2. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites.

- PLS-DA results:

In the left upper corner the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the upper right corner are represented the important features of the component with higher percentage of explained variability (in this case component 1) . Colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study. In the lower image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))


Scores Plot
adrenal
extraadrenal
Component 2 (11.1 %)
Component 1 (26.7 %)

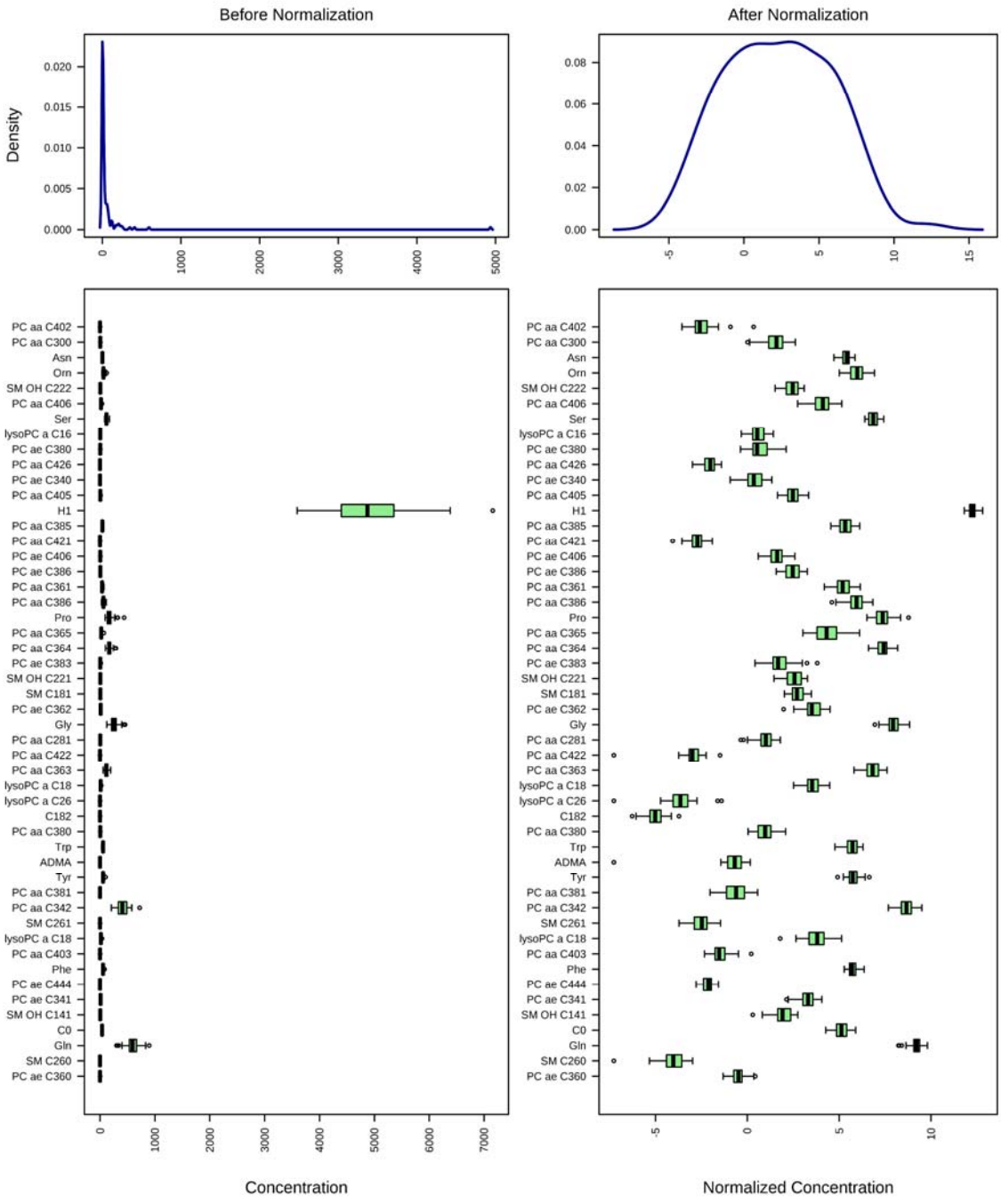

adrenal
extraadrenal
lysoPC a C260
PC ae C302
ADMA
Thr
C182
Glu
PC aa C381
PC ae C422
SM C161
SM OH C141
Trp
PC ae C405
SM OH C222
PC aa C322
PC ae C404
High
Low
1.5
2.0
2.5
3.0
3.5
VIP scores


Accuracy
R2
Q2
Performance
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
Number of components

2.1 c) Statistical analyses for catecholamine phenotype (adrenergic/noradrenergic) in PPGL patients prior surgery

- Normalization Result (56 preoperative patients/samples):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



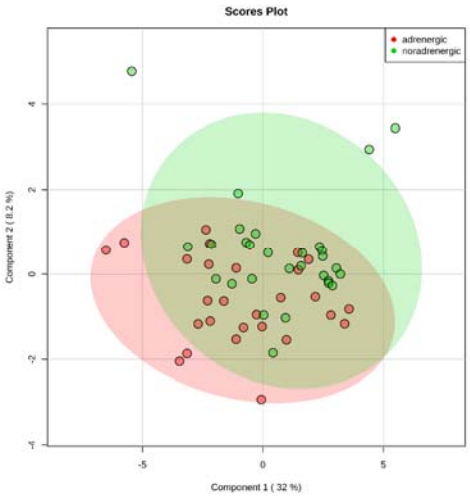
- Results of the performed statistical tests:

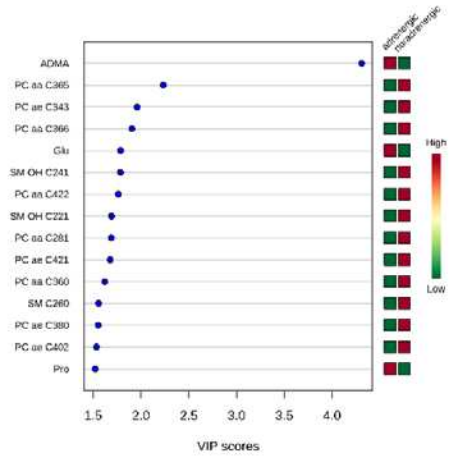
Metabolites (first 10) identifed by t-test analysis (none resulted significant after correction for FDR)						***Metabolites identified by fold change analysis (adren. VS noradrenergic)***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Fold Change**	**log2(FC)**
SM OH C221	-2.1335	0.03744	1.4267	0.75028		Glu	1.2639	0.33786
SM OH C241	-2.1113	0.03939	1.4046	0.75028		ADMA	1.1942	0.25608
PC aa C426	-2.0757	0.042698	1.3696	0.75028		Pro	1.1916	0.25286
PC aa C281	-2.0132	0.049092	1.309	0.75028		SM OH C241	0.84342	-0.24568
PC aa C360	-1.9365	0.058048	1.2362	0.75028		SM OH C221	0.84459	-0.24367
Pro	1.8967	0.063223	1.1991	0.75028		PC aa C365	0.8495	-0.23532
ADMA	1.8195	0.074386	1.1285	0.75028		PC ae C343	0.85115	-0.23251
Creatinine	-1.8136	0.075299	1.1232	0.75028		PC aa C366	0.85227	-0.23061
His	-1.7973	0.077884	1.1086	0.75028		PC aa C360	0.85858	-0.21998
PC aa C365	-1.7603	0.084017	1.0756	0.75028		PC aa C281	0.86099	-0.21594
Metabolites identified by EBAM								
none								
Metabolites identified by SAM								
Name	**d.value**	**stdev**	**rawp**	**q.value**				
none								

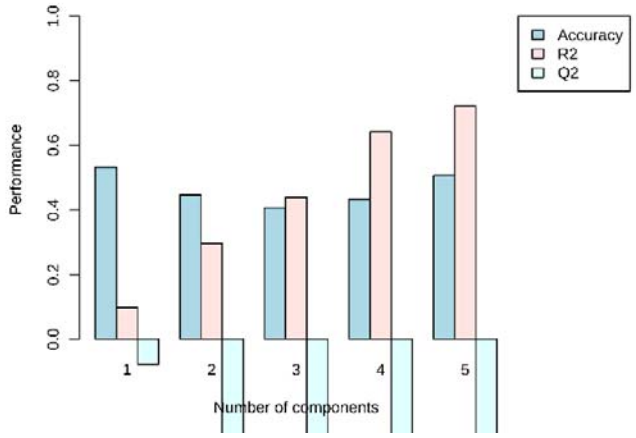
For the fold change analysis the fold change threshold was set at 1.15. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites.

- PLS-DA results:

In the left upper corner the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the upper right corner are represented the important features of the component with higher percentage of explained variability (in this case component 1). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study. In the lower image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))

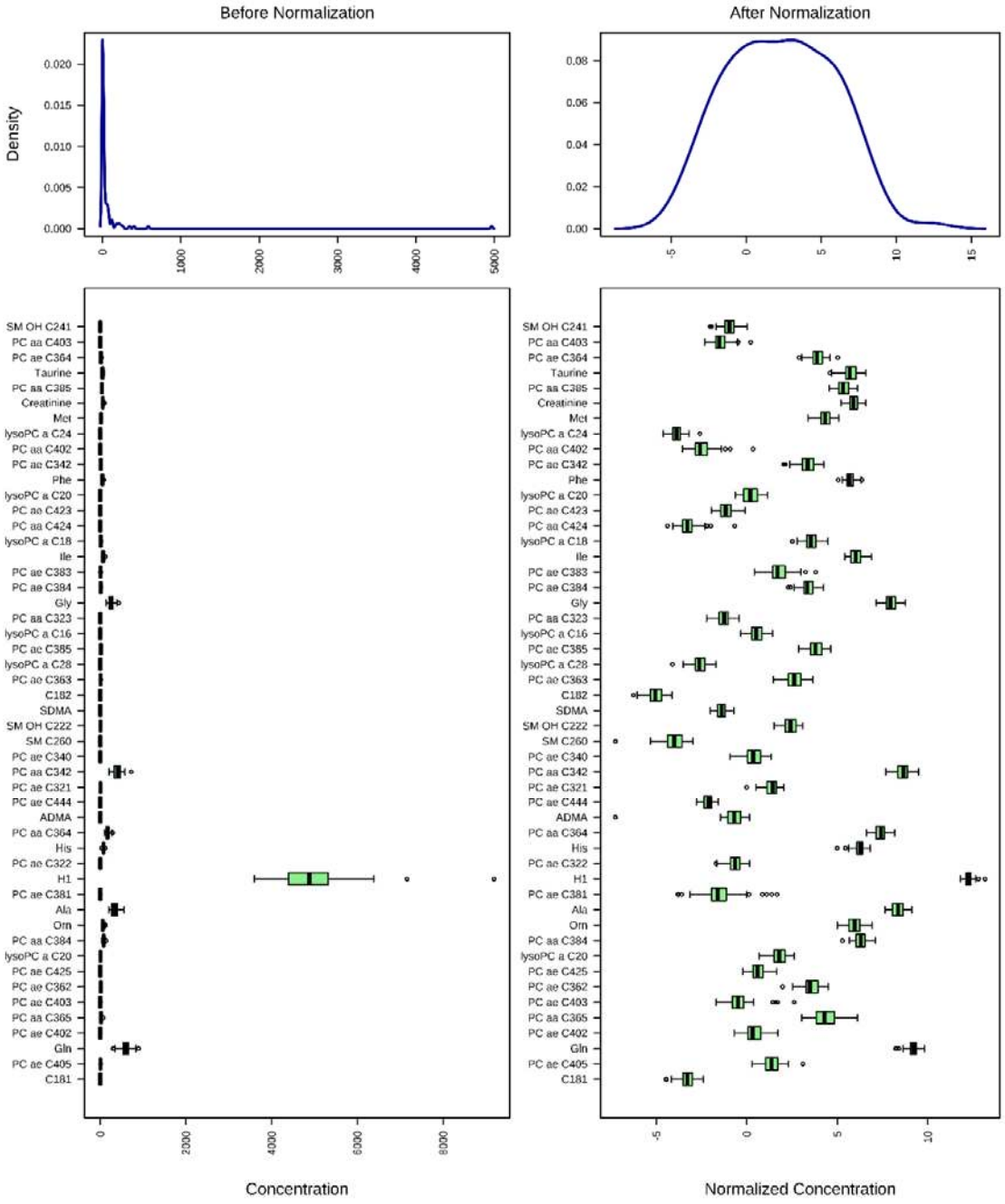

Scores Plot
adrenergic
noradrenergic
Component 2 (8.2 %)
Component 1 (32 %)


ADMA
PC aa C365
PC ae C343
PC aa C366
Glu
SM OH C241
PC aa C422
SM OH C221
PC aa C281
PC ae C421
PC aa C360
SM C260
PC ae C380
PC ae C402
Pro
adrenergic
noradrenergic
High
Low
1.5
2.0
2.5
3.0
3.5
4.0
VIP scores


Accuracy
R2
Q2
Performance
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
Number of components

2.1 d) Statistical analyses stratified by BMI (threshold 25kg/m2) in PPGL patients prior surgery

- Normalization Result (52 preoperative samples; 4 samples with BMI missing information excluded):



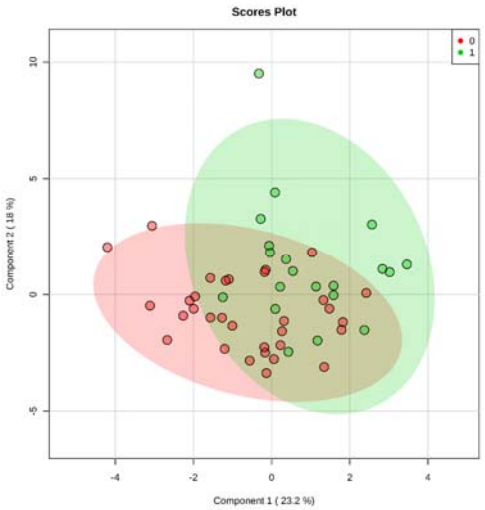
- Results of the performed statistical tests:

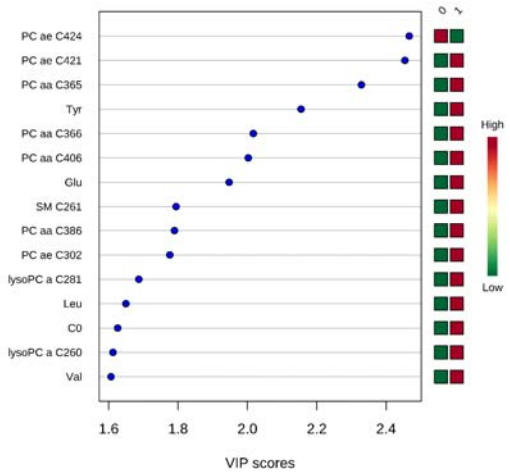
***Significant Metabolites identifed by* t-test analysis**						***Metabolites identified by fold change analysis (<25kg/m2 VS ≥25kg/m2)***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Fold**	**log2(FC)**
Tyr	-3.8	0.00039363	3.4049	0.04881		lysoPC a C260	0.76637	-0.38388
Significant metabolites identified by EBAM						PC aa C365	0.77996	-0.35852
Name	**z.value**	**posterior**	**local.fdr**			Glu	0.78329	-0.35239
none						PC aa C402	0.78909	-0.34173
						Tyr	0.80951	-0.30489
Significant metabolites identified by SAM						PC aa C424	0.81351	-0.29777
none						PC aa C366	0.82962	-0.26948
						C0	0.83136	-0.26645

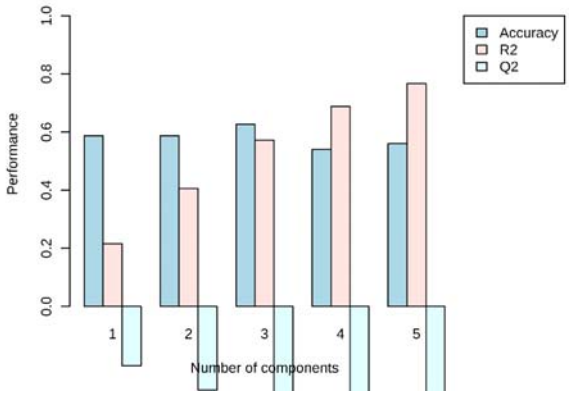
For the fold change analysis the fold change threshold was set at 1.20. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites/metabolites.

- PLS-DA results:

On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 1). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite in patients with BMI <25 kg/m2 (“0”) and ≥ 25 kg/m2 (“1”) before surgery. In the right image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))

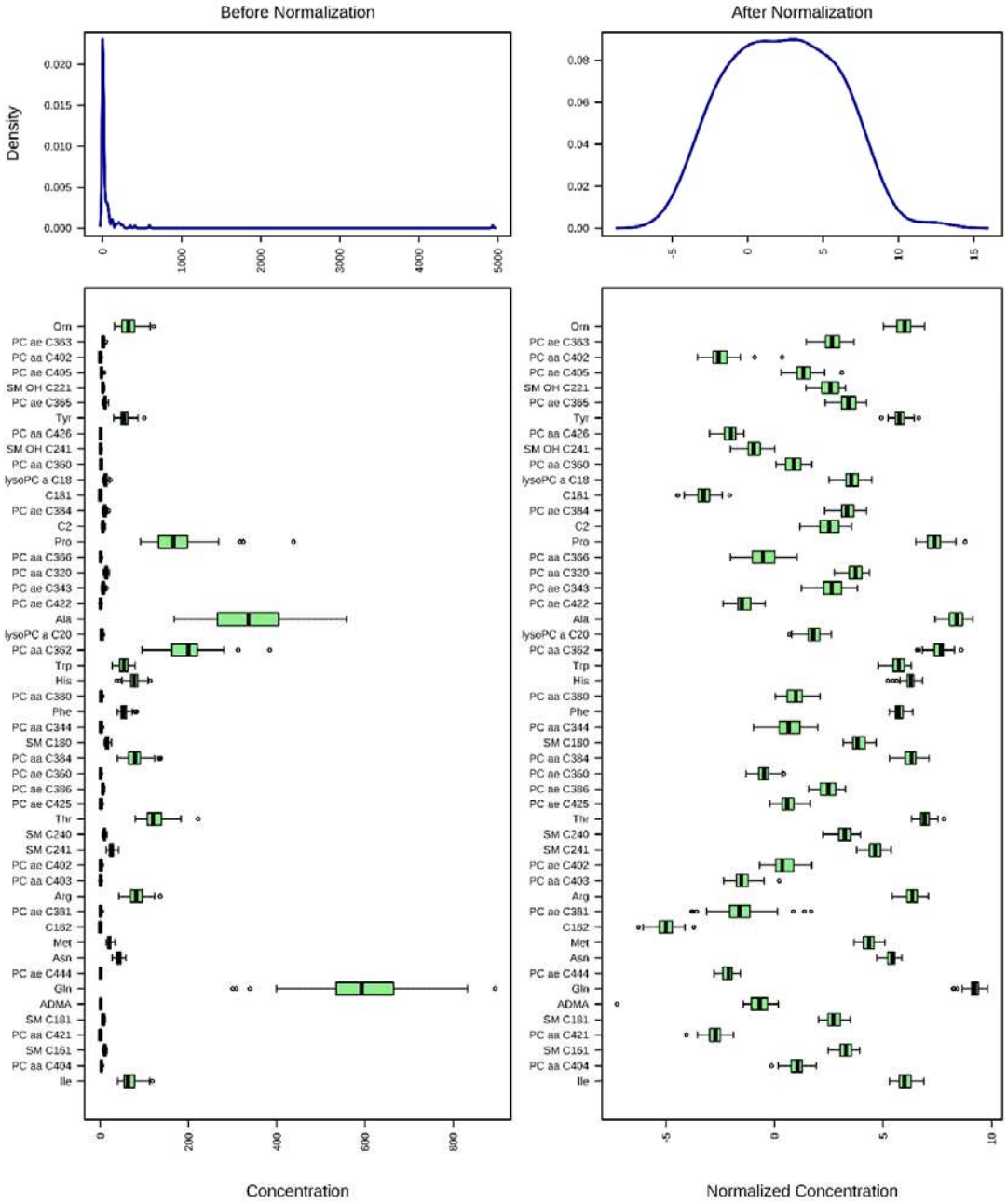

Scores Plot
0
1
Component 2 (18 %)
Component 1 (23.2 %)


PC ae C424
PC ae C421
PC aa C365
Tyr
PC aa C366
PC aa C406
Glu
SM C261
PC aa C386
PC ae C302
lysoPC a C281
Leu
C0
lysoPC a C260
Val
High
Low
VIP scores


Accuracy
R2
Q2
Performance
Number of components

2.1 e) Statistical analyses stratified by arterial hypertension (AHT) (present/absent) in PPGL patients prior surgery

- Normalization Result (56 preoperative samples):



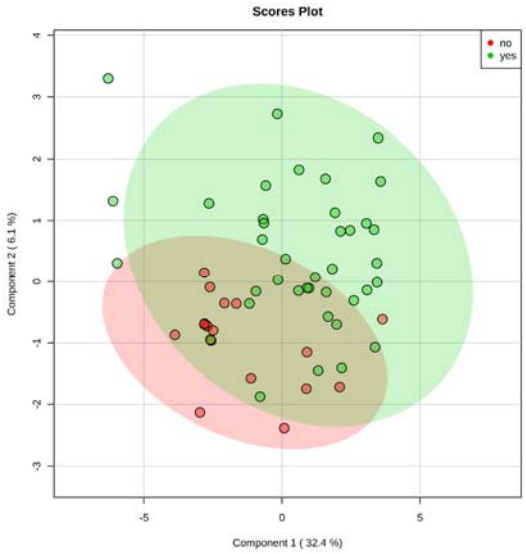
- Results of the performed statistical tests:

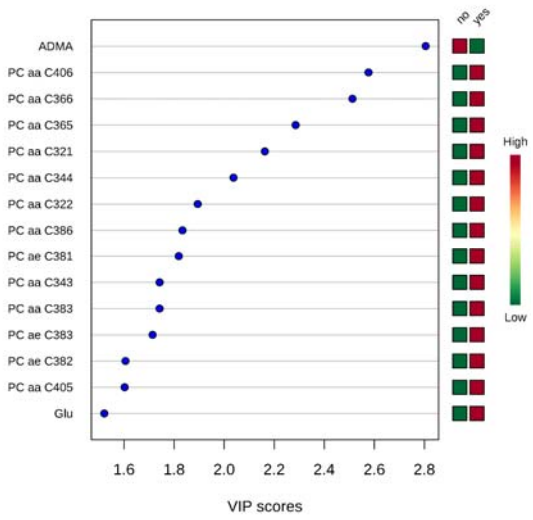
Metabolites (first 10) identifed by t-test analysis (none resulted significant after correction for FDR)						***Metabolites identified by fold change analysis (AHT yes VS no)***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Fold Change**	**log2(FC)**
PC aa C406	-3.0336	0.0037101	2.4306	0.46005		PC ae C381	1.6567	0.72828
PC aa C405	-2.597	0.012092	1.9175	0.52522		PC ae C403	1.4443	0.53041
PC aa C383	-2.5165	0.014854	1.8282	0.52522		PC aa C321	1.3776	0.46216
PC aa C366	-2.2408	0.029176	1.535	0.52522		PC aa C406	1.3747	0.45912
PC aa C321	-2.2101	0.031357	1.5037	0.52522		PC aa C366	1.3333	0.41504
PC aa C384	-2.1942	0.032546	1.4875	0.52522		PC ae C383	1.3282	0.40943
PC aa C386	-2.1643	0.034878	1.4574	0.52522		Glu	1.3222	0.40298
PC aa C343	-2.1199	0.038627	1.4131	0.52522		PC ae C382	1.3189	0.39932
PC aa C385	-2.0534	0.044893	1.3478	0.52522		PC aa C344	1.3154	0.39546
PC aa C425	-2.0384	0.046415	1.3333	0.52522		PC aa C424	1.2998	0.37828
Significant metabolites identified by EBAM						PC aa C322	1.2923	0.36994
none						PC aa C402	1.2802	0.35637
Metabolites identified by SAM (not significant after correction						PC aa C343	1.2757	0.35126
Name	**d.value**	**stdev**	**rawp**	**q.value**		PC aa C365	1.2585	0.33176
none								

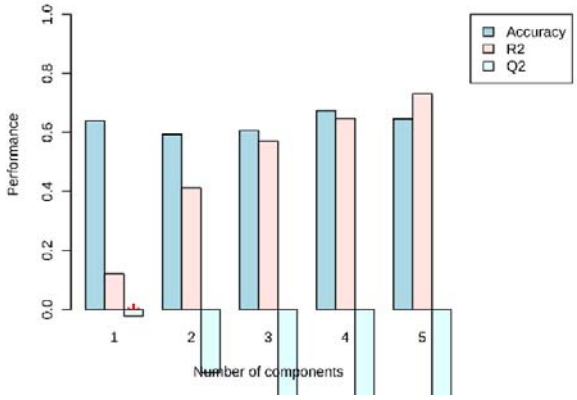
For the fold change analysis the fold change threshold was set at 1.25.
Abbreviations: FDR, false discovery rate; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites.

- PLS-DA results:

On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 2) . Colored boxes on the right indicate the relative concentrations of the corresponding metabolite in patients with (yes) and without (no) arterial hypertension before surgery. In the right image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))

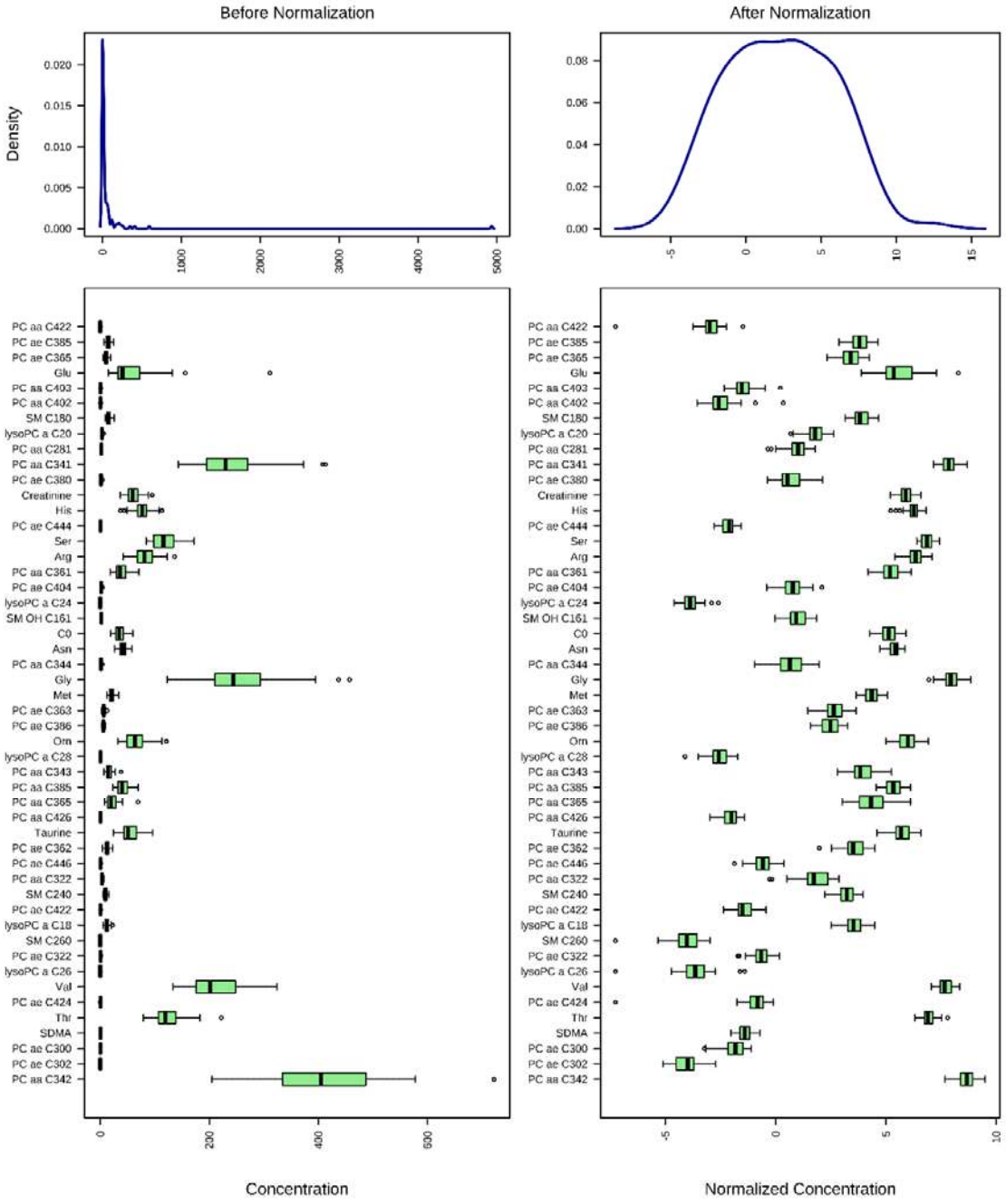

Scores Plot
no
yes
Component 2 (6.1 %)
Component 1 (32.4 %)


no
yes
ADMA
PC aa C406
PC aa C366
PC aa C365
PC aa C321
PC aa C344
PC aa C322
PC aa C386
PC ae C381
PC aa C343
PC aa C383
PC ae C383
PC ae C382
PC aa C405
Glu
High
Low
1.6
1.8
2.0
2.2
2.4
2.6
2.8
VIP scores


Accuracy
R2
Q2
Performance
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
Number of components

2.1 f) Statistical analyses stratified by diabetes mellitus (present/absent) in PPGL patients prior surgery

- Normalization Result (56 preoperative samples):



- Results of the performed statistical tests:

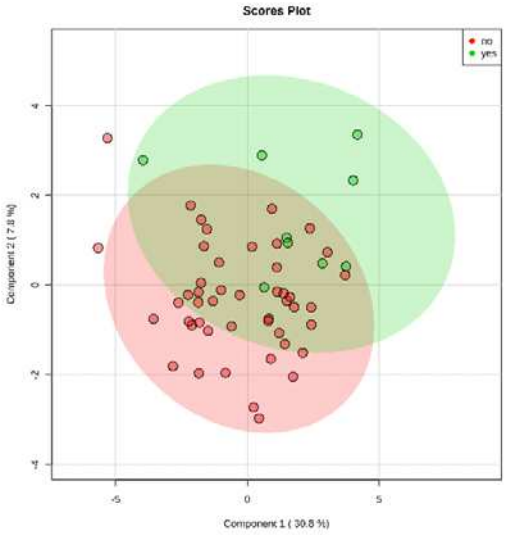
Metabolites (first 10) identifed by t-test analysis (none resulted significant after correction for FDR)						***Metabolites identified by fold change analysis (diabetes mellitus yes VS no)***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Fold Change**	**log2(FC)**
Cit	3.3777	0.0013622	2.8658	0.16162		Cit	0.74864	-0.41766
lysoPC a C160	-3.1571	0.0026068	2.5839	0.16162		lysoPC a C160	1.3303	0.41175
H1	-2.9691	0.0044477	2.3519	0.18384		lysoPC a C181	1.3175	0.39784
Thr	2.8216	0.0066726	2.1757	0.20685		lysoPC a C182	1.3158	0.39597
lysoPC a C181	-2.4825	0.016187	1.7908	0.40144		lysoPC a C170	1.2645	0.33862
lysoPC a C180	-2.071	0.043157	1.3649	0.71341		lysoPC a C180	1.2543	0.32685
lysoPC a C170	-2.0576	0.044471	1.3519	0.71341		PC aa C386	1.2442	0.31522
C182	-2.0174	0.048635	1.3131	0.71341		lysoPC a C161	1.2366	0.30633
lysoPC a C182	-1.8771	0.065909	1.1811	0.71341		Thr	0.8102	-0.30366
lysoPC a C161	-1.8596	0.068394	1.165	0.71341		lysoPC a C204	1.2274	0.29561
Significant metabolites identified by EBAM						C182	1.2184	0.28499
Name	**z.value**	**posterior**	**local.fdr**			lysoPC a C203	1.2035	0.2672
none								
Metabolites identified by SAM								
Name	**d.value**	**stdev**	**rawp**	**q.value**				
none								

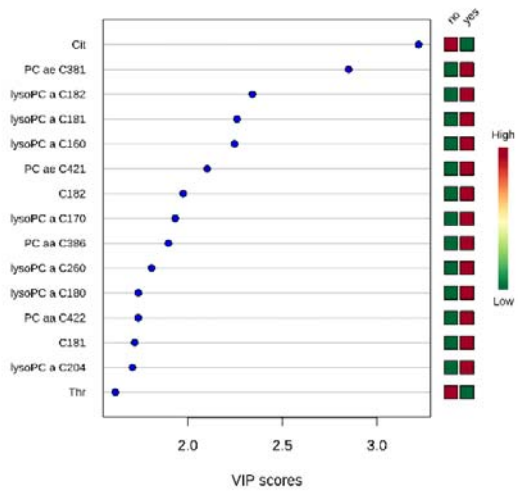
For the fold change analysis the fold change threshold was set at 1.20

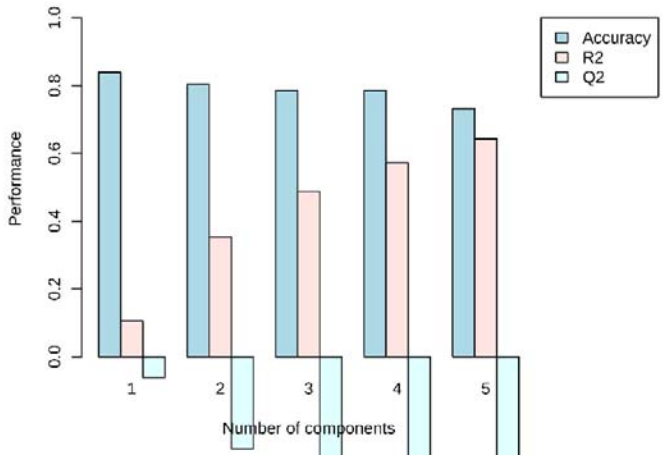
Abbreviations: FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites.

- PLS-DA results:

On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 1). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite in patients with (yes) and without (no) diabetes mellitus before surgery. In the right image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))

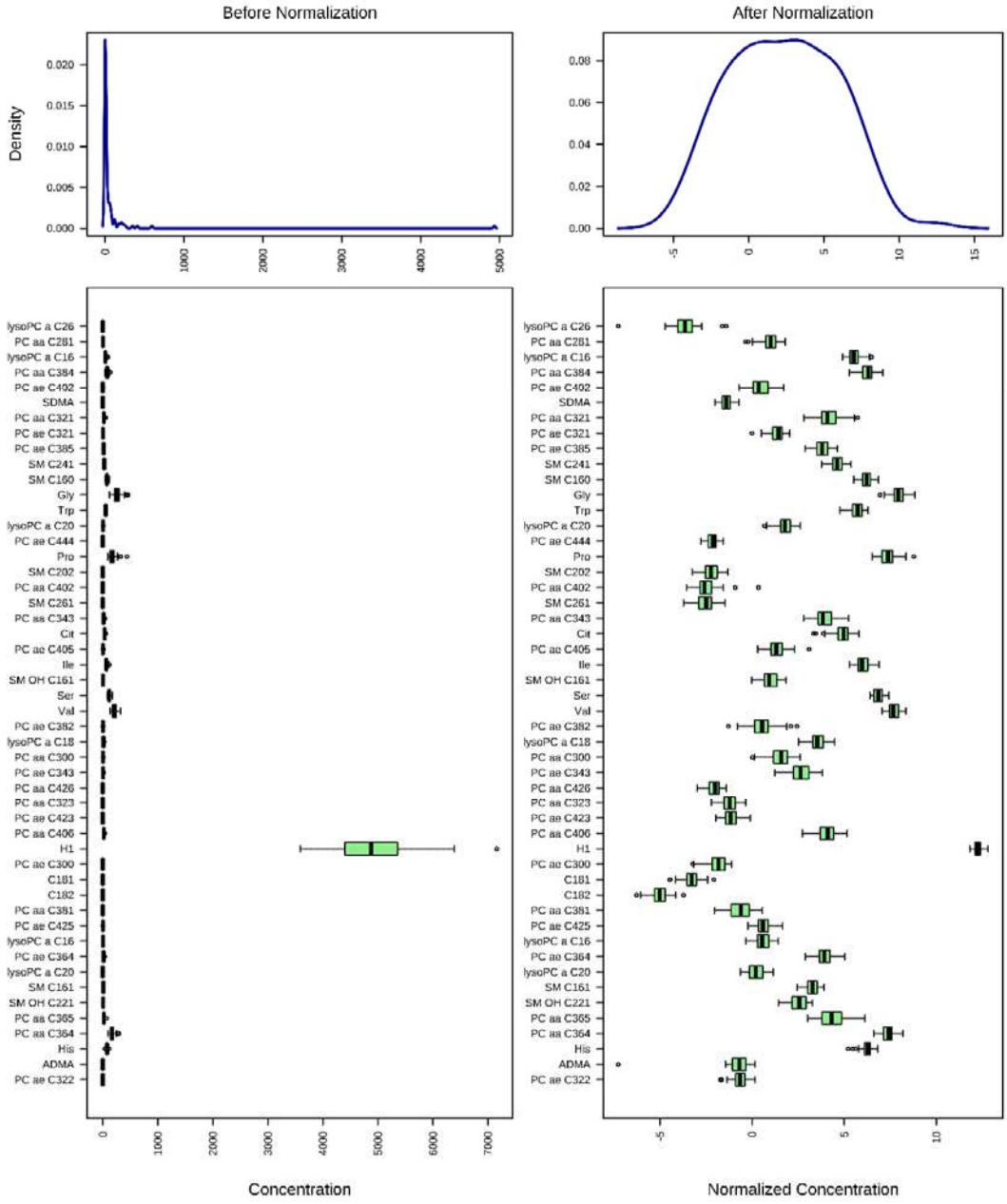

Scores Plot
no
yes
Component 2 (7.8 %)
Component 1 (30.8 %)


no
yes
Cit
PC ae C381
lysoPC a C182
lysoPC a C181
lysoPC a C160
PC ae C421
C182
lysoPC a C170
PC aa C386
lysoPC a C260
lysoPC a C180
PC aa C422
C181
lysoPC a C204
Thr
High
Low
2.0
2.5
3.0
VIP scores


Accuracy
R2
Q2
Performance
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
Number of components

2.1 g) Statistical analyses stratified by age (threshold 45 years) in PPGL patients prior surgery

- Normalization Result (56 preoperative samples):



- Results of the performed statistical tests:

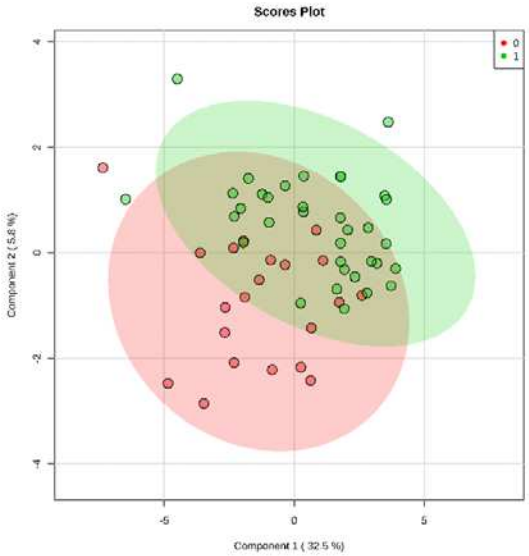
Metabolites (first 10) identifed by t-test analysis (none resulted significant after correction for FDR)						***Metabolites identified by fold change analysis (age <45 no VS ≥45)***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Fold Change**	**log2(FC)**
SM C181	-3.0793	0.0032592	2.4869	0.12698		Glu	0.71349	-0.48703
PC aa C406	-3.0115	0.0039495	2.4035	0.12698		C2	0.7431	-0.42836
C181	-2.9679	0.0044625	2.3504	0.12698		SM C261	0.76498	-0.38651
SM C161	-2.9361	0.0048752	2.312	0.12698		PC aa C406	0.77049	-0.37616
Met	2.8416	0.0063204	2.1993	0.12698		C182	0.772	-0.37333
C182	-2.8001	0.007073	2.1504	0.12698		C181	0.77481	-0.36808
PC aa C384	-2.7951	0.0071682	2.1446	0.12698		PC aa C366	0.78602	-0.34737
SM C261	-2.7265	0.0086144	2.0648	0.13352		PC ae C302	0.79172	-0.33694
Trp	2.622	0.011334	1.9456	0.14073		lysoPC a C260	0.79394	-0.33289
SM C202	-2.6215	0.011349	1.945	0.14073		PC ae C402	0.7966	-0.32807
Significant metabolites identified by EBAM						PC ae C421	0.79716	-0.32705
Name	**z.value**	**posterior**	**local.fdr**					
SM C181	3.0793	0.96146	0.038543					
PC aa C406	3.0115	0.95929	0.04071					
C181	2.9679	0.95783	0.042168					
SM C161	2.9361	0.95673	0.043267					
C182	2.8001	0.95166	0.04834					
PC aa C384	2.7951	0.95146	0.048537					
Metabolites identified by SAM								
Name	**d.value**	**stdev**	**rawp**	**q.value**				
none								

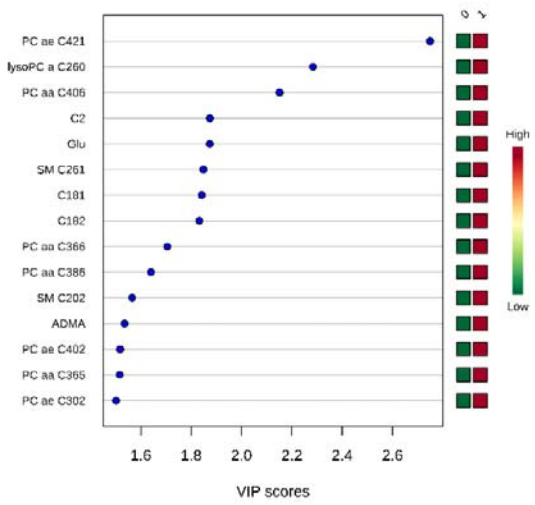
For the fold change analysis the fold change threshold was set at 1.25. For EBAM the delta value was set at 0.95 with an FDR of 0.044

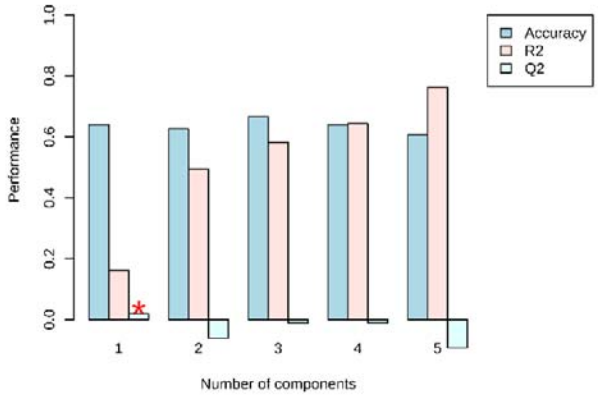
Abbreviations: FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites.

- PLS-DA results:

On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 1). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite in patients with age <45 years (“0”) and ≥ 45 years (“1”) before surgery. In the right image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))


Scores Plot
0
1
Component 2 (5.8 %)
Component 1 (32.5 %)


PC ae C421
lysoPC a C260
PC aa C406
C2
Glu
SM C261
C181
C182
PC aa C366
PC aa C386
SM C202
ADMA
PC ae C402
PC aa C365
PC ae C302
High
Low
VIP scores


Accuracy
R2
Q2
Performance
Number of components

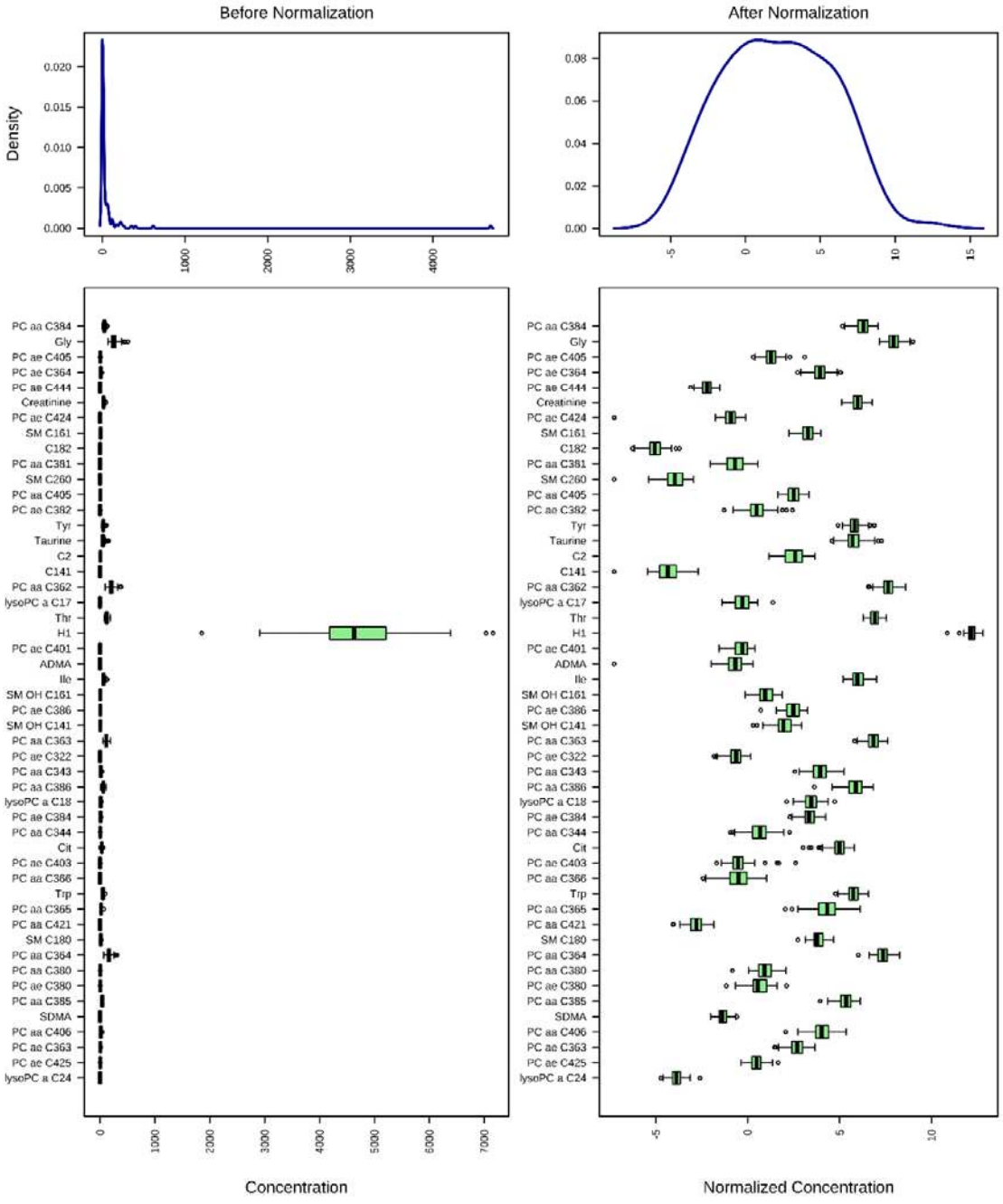
2.2 Changes of targeted metabolomics following surgery (considering only the patients with biochemical remission following surgery):

- all patients (a), female (b) and male (c), as well as adrenergic (d) and noradrenergic (e) tumor patients separately

2.2 a) Statistical analysis for comparison before and after surgery in all patients with biochemical remission

- Normalization Result (106 samples, 53 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



- Results of the performed statistical tests:

Significant metabolites identifed by paired t-tests			
Name (Abbreviation)	**t.stat**	**p.value**	**FDR**
PC aa C420	4.0406	0.0001765	3.7533
His	-4.0121	0.00019353	3.7133
H1	3.5508	0.00082543	3.0833
PC ae C445	3.4894	0.00099481	3.0023
Creatinine	-3.3712	0.0014182	2.8483
PC ae C446	3.2753	0.0018821	2.7254

Significant metabolites identified by SAM				
Name	**d.value**	**stdev**	**rawp**	**q.value**
PC aa C420	-2.6005	0.048767	0.00023622	0.020665
His	2.386	0.039623	0.0007874	0.034441
H1	-2.2221	0.045165	0.0012598	0.036737
PC aa C386	-2.0087	0.05566	0.0029134	0.054245
PC ae C445	-1.9649	0.034807	0.0034646	0.054245
PC ae C446	-1.9062	0.037601	0.0044882	0.054245
PC ae C425	-1.9059	0.044368	0.0044882	0.054245
PC aa C421	-1.7738	0.046891	0.0080315	0.076397

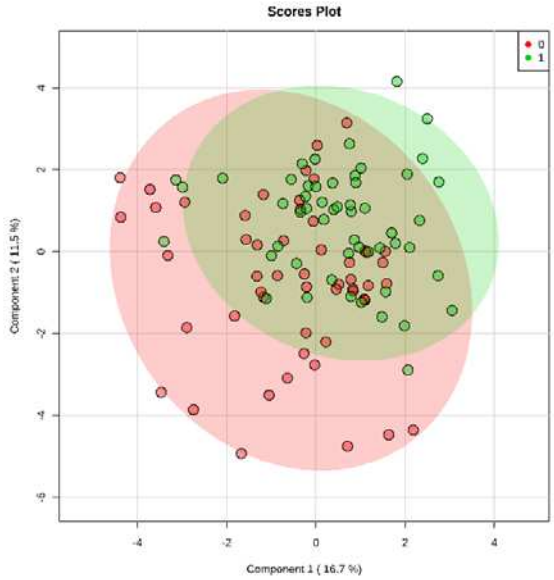
Significant metabolites identified by fold change analysis (paired)			
Name (Abbreviation)	**Count (up)**	**Count (down)**	
PC aa C420	30	7	
His	7	26	
PC ae C425	26	7	
H1	27	8	
PC ae C445	26	9	
Orn	13	28	
lysoPC a C180	12	27	
PC ae C446	26	11	
SM C261	28	13	
Sarcosine	15	29	
Taurine	12	26	
PC aa C321	28	16	
C141	29	18	
SM C260	17	26	
ADMA	19	26	
PC ae C301	28	21	
PC ae C381	28	21	
lysoPC a C260	22	26	

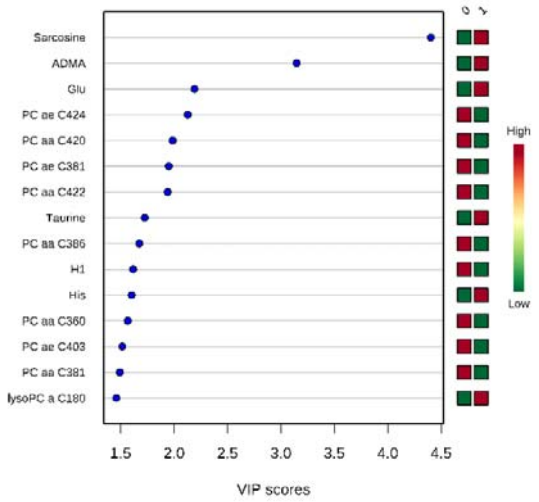
Significant metabolites identified by EBAM				
Name	z.value	posterior	local.fdr	
PC aa C420	-4.0406	0.99019	0.0098134	
His	4.0121	0.96325	0.036749	
H1	-3.5508	0.97099	0.029005	
PC ae C445	-3.4894	0.9669	0.033103	
Creatinine	3.3712	0.93726	0.06274	
PC ae C446	-3.2753	0.94813	0.051869	
PC ae C425	-3.066	0.92132	0.078684	

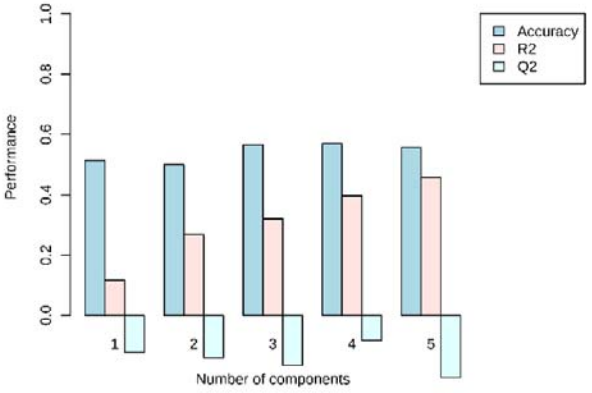
Notes: For the fold change analysis the fold change threshold was set at 1.1 with a cut-off of 50% of pairs and the results represent the number of pairs with upregulated (count up) or downregulated (count down) values prior surgery. For SAM analysis the delta value was at 0.7 for an FDR value of 0.044; the q.value represent the adjusted p.value for multiple analysis. For EBAM the delta value was set at 0.92 with an FDR of 0.043.
Abbreviations: FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites.

- PLS-DA results:

On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 1). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before ("0") and after ("1") surgery. In the right image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))



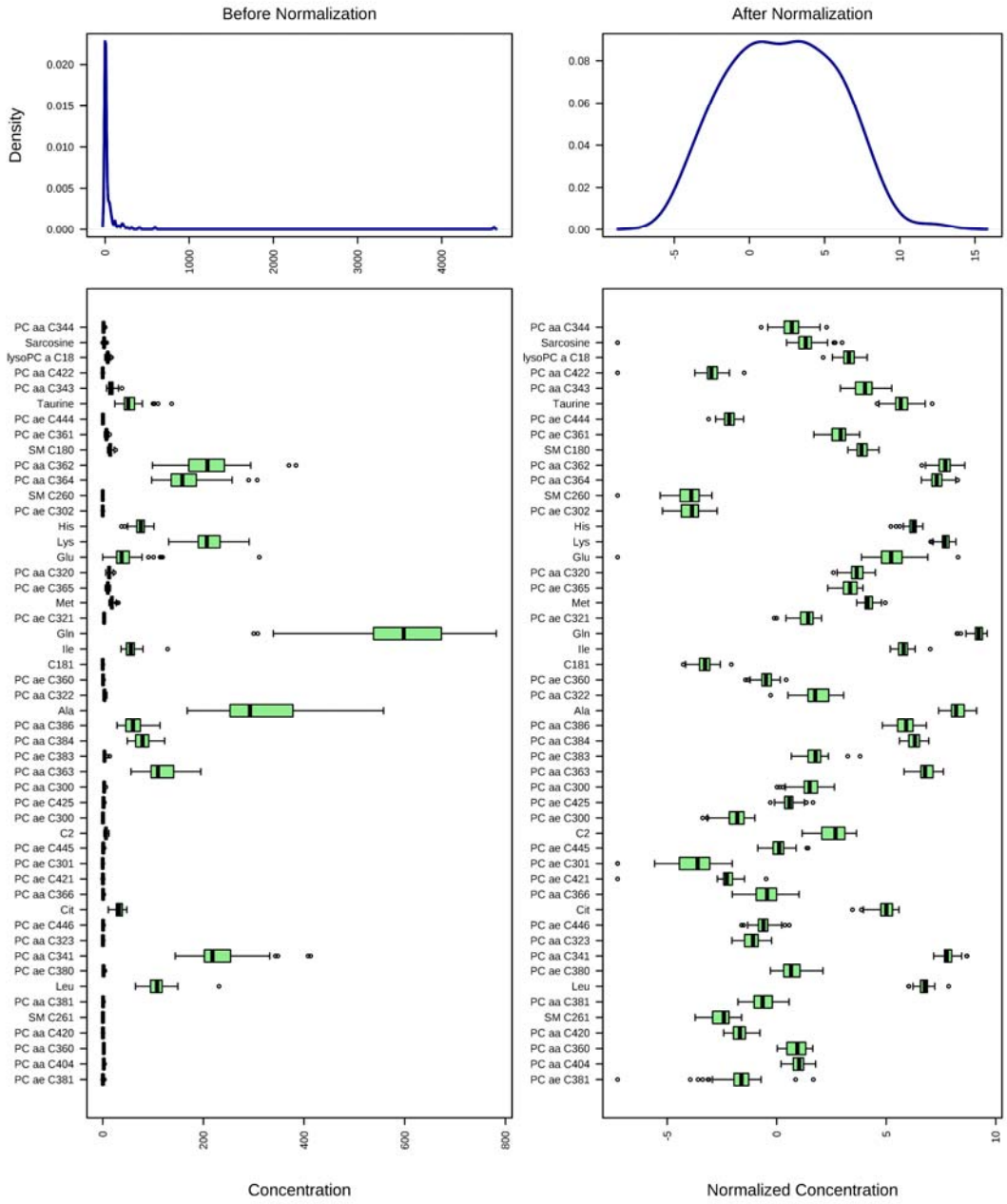




2.2 b) Statistical analysis for comparison before and after surgery in female patients with biochemical remission

- Normalization Result (62 samples, 31 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



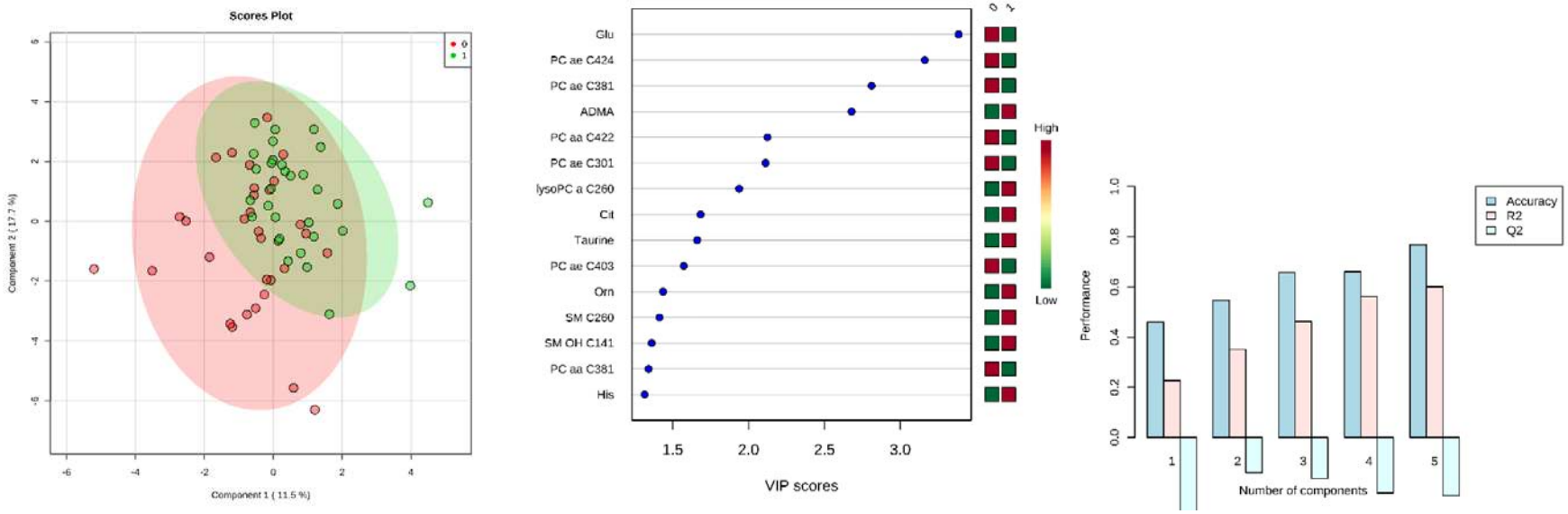
- Results of the performed statistical tests:

Metabolites (first 10) identifed by paired t-tests (none resulted significant after correction for FDR)						***Significant metabolites identified by fold change analysis***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Count (up)**	**Count (down)**
Cit	-3.2032	0.0032121	2.4932	0.14001		His	5	17
His	-3.1146	0.004032	2.3945	0.14001		PC aa C321	19	7
H1	3.0683	0.0045355	2.3434	0.14001		Gln	5	16
Gln	-3.0424	0.004843	2.3149	0.14001		Cit	7	17
PC aa C420	2.9485	0.0061327	2.2123	0.14001		C0	7	16
SM OH C141	-2.9181	0.0066148	2.1795	0.14001		Orn	7	16
PC ae C364	-2.8396	0.008033	2.0951	0.14574		Sarcosine	8	17
Val	-2.7556	0.009865	2.0059	0.1554		SM C260	9	18
PC aa C362	-2.6741	0.012008	1.9205	0.1554		Leu	8	16
PC aa C386	2.6663	0.012236	1.9124	0.1554		C141	17	10
Metabolites (first 10) identified by SAM (none resulted significant after correction for FDR)						lysoPC a C260	11	18
Name	**d.value**	**stdev**	**rawp**	**q.value**		PC aa C300	16	10
Cit	2.1302	0.065794	0.0029134	0.11734		PC ae C381	17	11
His	2.0116	0.060441	0.0040945	0.11734		PC ae C301	16	11
Gln	1.8222	0.049488	0.0072441	0.11734				
PC ae C364	1.7558	0.053691	0.0094488	0.11734				
Val	1.7388	0.056675	0.010394	0.11734				
Tyr	1.6533	0.060283	0.014331	0.1197				
PC aa C362	1.5958	0.049043	0.01874	0.1197				
Orn	1.5926	0.074848	0.018976	0.1197				
SM OH C141	1.5013	0.035115	0.028346	0.13734				
PC ae C342	1.4831	0.057917	0.030236	0.13734				
Significant metabolites identified by EBAM								
Name	**z.value**	**posterior**	**local.fdr**					
none								

Notes: For the fold change analysis the fold change threshold was set at 1.1 with a cut-off of 50% of pairs; the numbers represent the number of pairs with upregulated (count up) or downregulated (count down) values prior surgery. For SAM the delta value was at 0.6 for an FDR value of 0.134; the q.value represent the FDR-adjusted p.value. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites

- PLS-DA results:

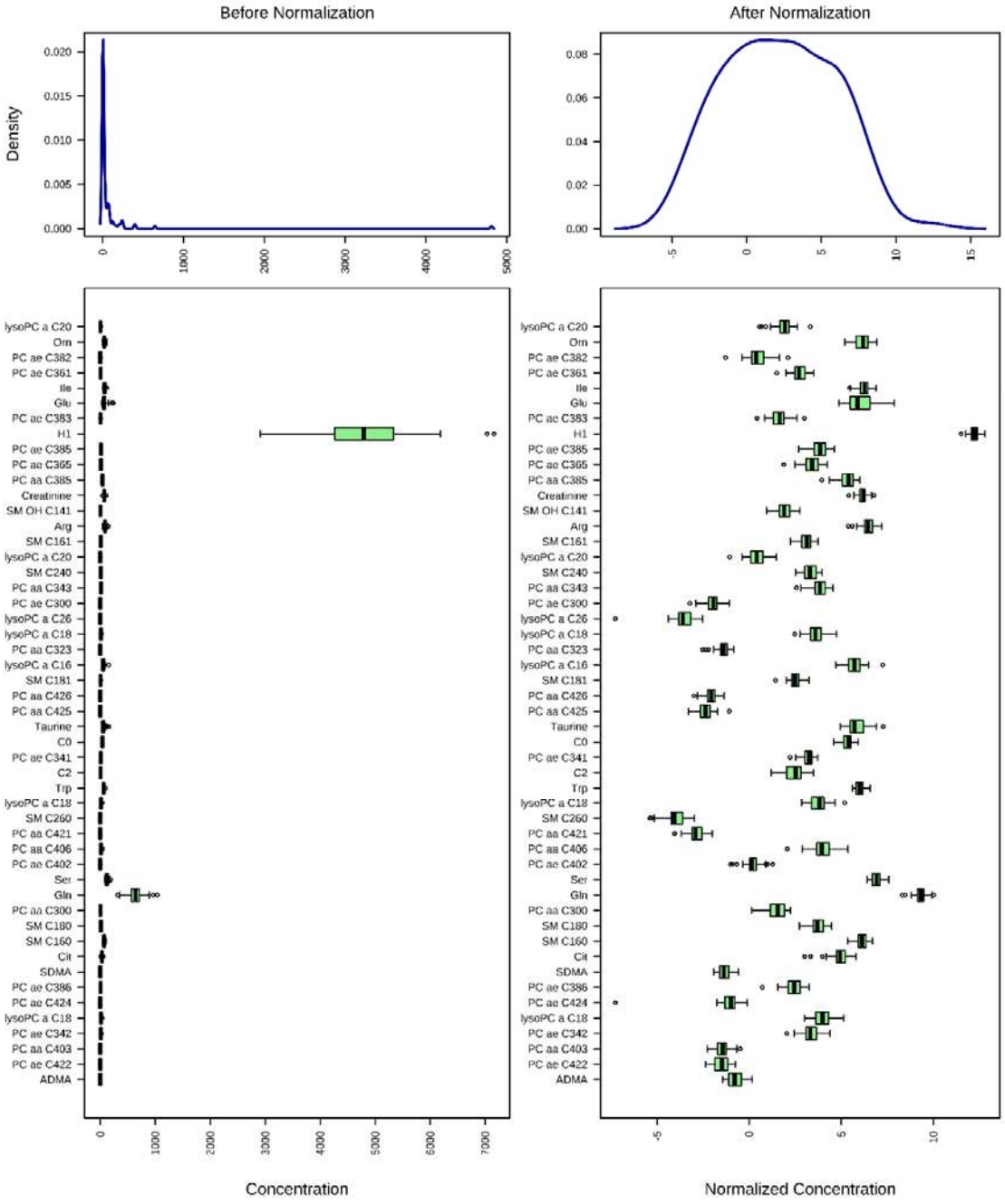
On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 2). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before ("0") and after ("1") surgery. In the right image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))



2.2 c) Statistical analysis for comparison before and after surgery in male patients with biochemical remission

- Normalization Result (44 samples, 22 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



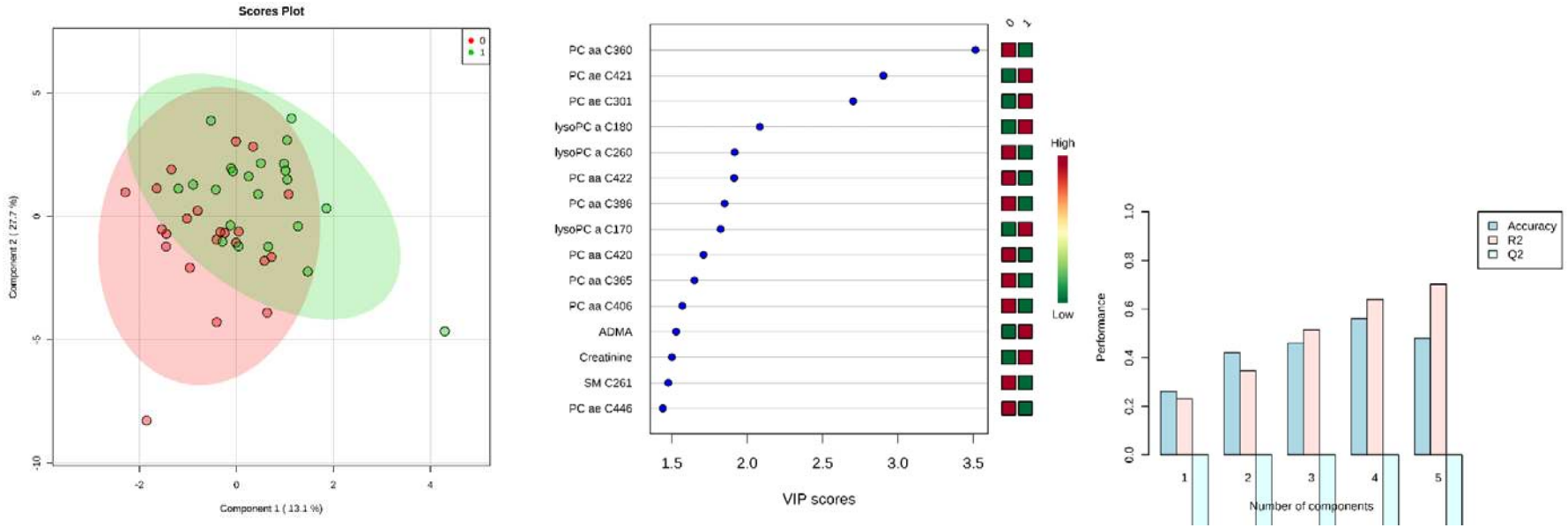
- Results of the performed statistical tests:

Metabolites (first 10) identifed by paired-t-test analysis (none resulted significant after correction for FDR)						***Metabolites identified by fold change analysis***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Count (up)**	**Count (down)**
Creatinine	-3.1501	0.0048311	2.316	0.49077		lysoPC a C180	4	13
His	-2.7199	0.01283	1.8918	0.49077		PC aa C420	13	4
PC aa C420	2.7108	0.013091	1.883	0.49077		H1	11	3
PC ae C446	2.6321	0.01558	1.8074	0.49077		ADMA	5	12
lysoPC a C180	-2.415	0.024941	1.6031	0.56157		lysoPC a C170	5	12
PC ae C445	2.3822	0.026741	1.5728	0.56157		Glu	7	12
PC ae C425	2.2029	0.038909	1.41	0.70036		Orn	6	11
SM C241	2.0787	0.050089	1.3003	0.70684		Pro	7	12
PC aa C361	-2.0106	0.057385	1.2412	0.70684		SM C261	11	6
PC aa C404	-1.8988	0.071422	1.1462	0.70684		PC aa C380	11	7
Metabolites identified by EBAM						C141	11	8
none						PC ae C381	11	8
Metabolites (first 10) identified by SAM (none resulted significant after correction for FDR)						PC ae C301	11	10
Name	**d.value**	**stdev**	**rawp**	**q.value**				
PC aa C420	-1.5434	0.077349	0.013095	0.29791				
Creatinine	1.5225	0.054728	0.014127	0.29791				
lysoPC a C180	1.4834	0.093157	0.016667	0.29791				
PC ae C446	-1.4053	0.067018	0.021508	0.29791				
PC ae C445	-1.2381	0.06331	0.039365	0.34769				
SM C241	-1.0916	0.064703	0.068016	0.34769				
H1	-1.0774	0.077016	0.072143	0.34769				
PC ae C425	-1.0579	0.054058	0.077063	0.34769				
PC aa C386	-1.0389	0.10429	0.082222	0.34769				
lysoPC a C260	-0.95163	0.1859	0.11302	0.38912				

Notes: For the fold change analysis the fold change threshold was set at 1.15 with a cut-off of 50% of pairs; the numbers represent the number of pairs with upregulated (count up) or downregulated (count down) values prior surgery. For SAM analysis the delta value was at 0.2 for an FDR value of 0.341; the q.value represent the FDR-adjusted p.value. For EBAM the delta value was set at 0.9 with an FDR of 0.0. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites

- PLS-DA results:

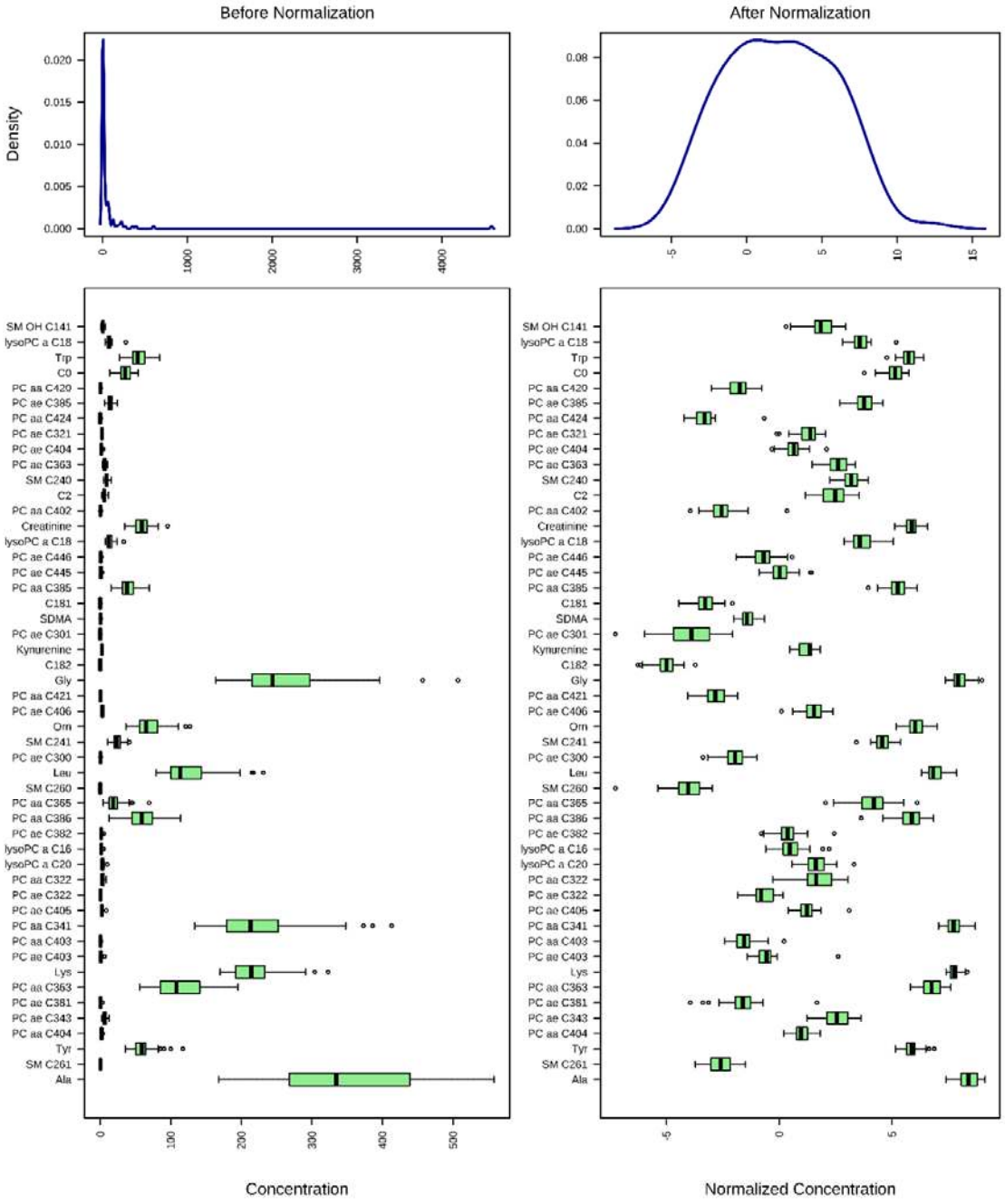
On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 2). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before ("0") and after ("1") surgery. In the right image the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))



2.2 d) Statistical analysis for comparison before and after surgery in adrenergic tumor patients with biochemical remission

- Normalization Result (50 samples, 25 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



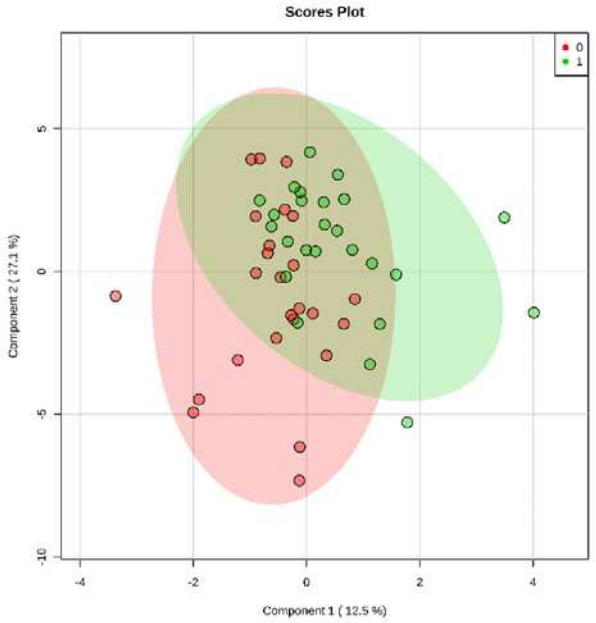
- Results of the performed statistical tests:

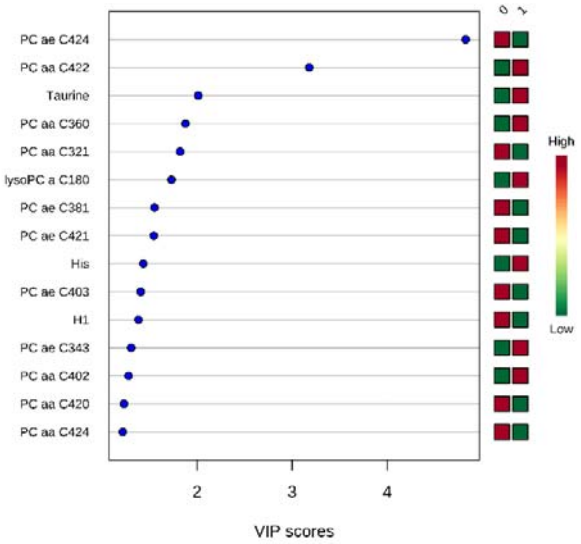
Metabolites (first 10) identifed by paired-t-test analysis (none resulted significant after correction for FDR)						***Metabolites identified by fold change analysis***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Count (up)**	**Count (down)**
His	-2.8386	0.0090758	2.0421	0.7671		PC aa C321	16	5
PC aa C321	2.4302	0.022936	1.6395	0.7671		lysoPC a C180	4	13
H1	2.3845	0.025356	1.5959	0.7671		Gln	5	13
PC aa C361	-2.2853	0.031423	1.5028	0.7671		PC ae C342	4	12
lysoPC a C180	-2.2359	0.034917	1.457	0.7671		ADMA	7	14
PC aa C362	-2.1434	0.042428	1.3723	0.7671		PC aa C386	12	5
SM C240	-2.0805	0.048324	1.3158	0.7671		PC aa C402	5	12
Taurine	-2.0707	0.049312	1.307	0.7671		SM C260	7	12
PC ae C445	2.001	0.056823	1.2455	0.7671		Glu	8	12
PC ae C446	1.8197	0.081302	1.0899	0.7671		lysoPC a C260	8	12
Metabolites identified by EBAM								
none								
Metabolites (first 10) identified by SAM (none resulted significant after correction for FDR)								
Name	**d.value**	**stdev**	**rawp**	**q.value**				
PC ae C424	-1.1694	0.35671	0.0064	0.50147				
PC aa C321	-1.0103	0.092516	0.01688	0.50147				
PC aa C422	0.96309	0.23253	0.0212	0.50147				
Taurine	0.9621	0.11284	0.02136	0.50147				
His	0.90922	0.06127	0.02728	0.50147				
lysoPC a C180	0.88969	0.085924	0.03024	0.50147				
H1	-0.83327	0.069843	0.04152	0.59017				
PC aa C420	-0.64611	0.080528	0.10728	0.65868				
PC ae C445	-0.61642	0.057883	0.12304	0.65868				
lysoPC a C204	-0.59994	0.080184	0.134	0.65868				

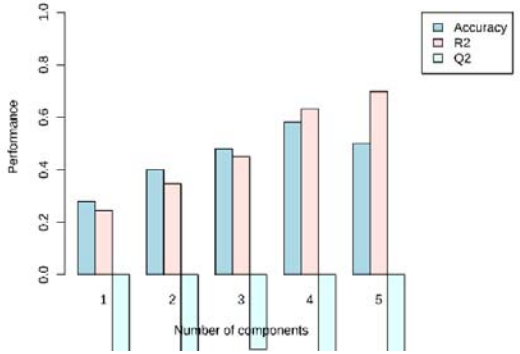
Notes: For the fold change analysis the fold change threshold was set at 1.15 with a cut-off of 50% of pairs; the numbers represent the number of pairs with upregulated (count up) or downregulated (count down) values prior surgery. For SAM analysis the delta value was at 0.14 for an FDR value of 0.537; the q.value represent the FDR-adjusted p.value. For EBAM the delta value was set at 0.9 with an FDR of 0.0. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites

- PLS-DA results:

On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 2). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before ("0") and after ("1") surgery. In the right image, the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))



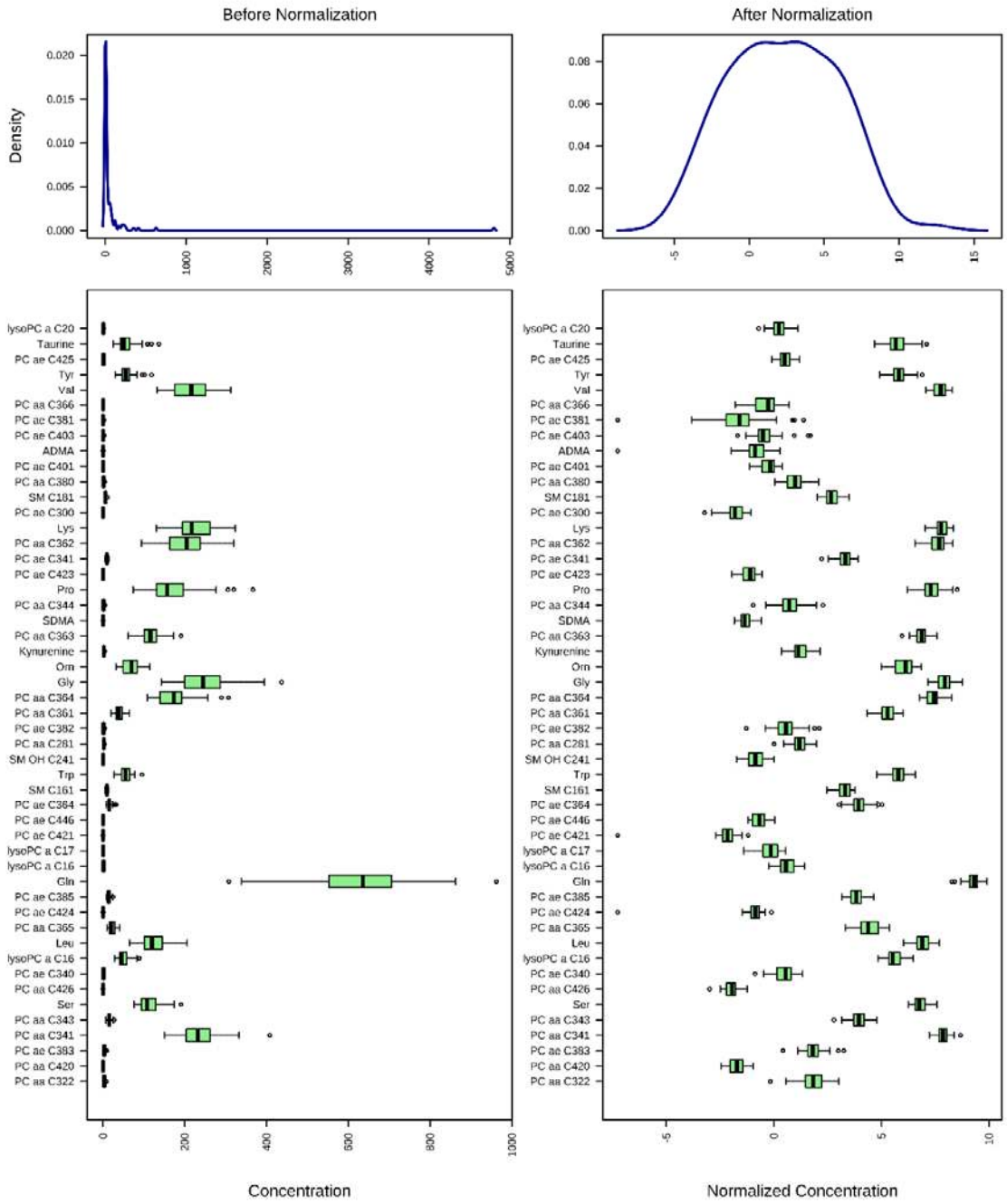




2.2 e) Statistical analysis for comparison before and after surgery in noradrenergic patients with biochemical remission

- Normalization Result (56 samples, 28 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



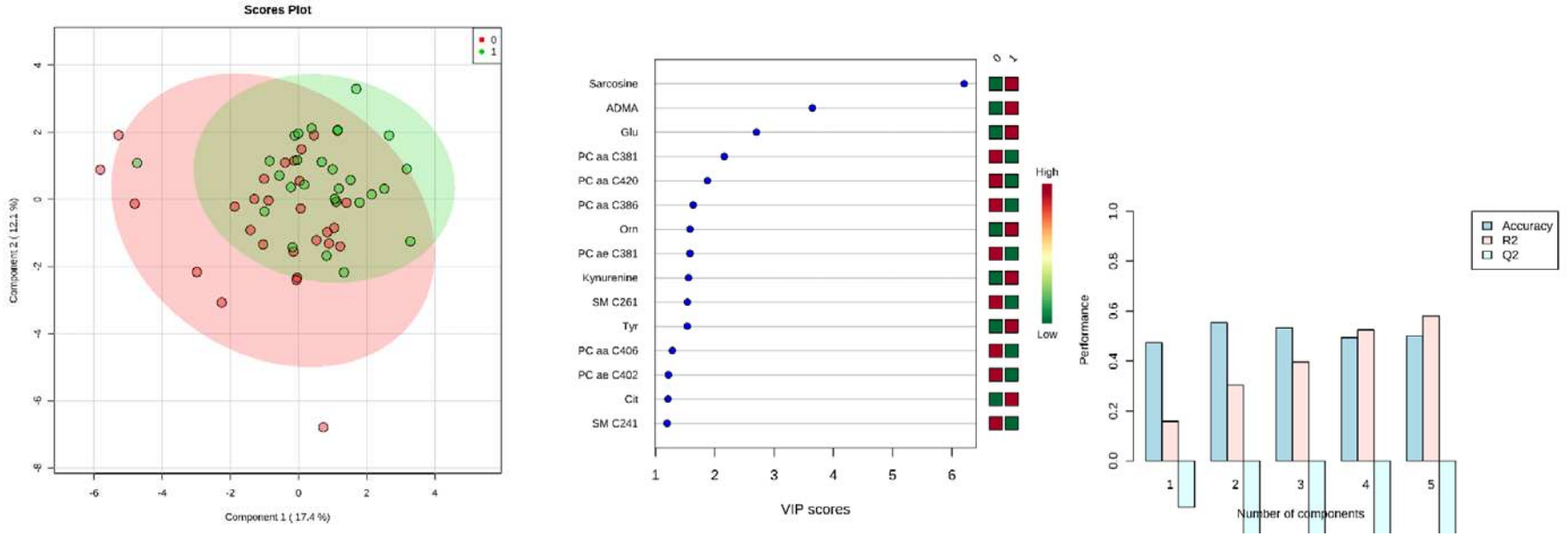
- Results of the performed statistical tests:

Significant metabolites identified by paired t-test analysis						***Significant metabolites identified by fold change***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Count (up)**	**Count (down)**
PC ae C425	4.4025	1.52E-04	3.8195	0.010002		PC aa C420	19	3
PC aa C420	4.3851	1.59E-04	3.7992	0.010002		PC ae C425	16	1
SM C241	3.7865	7.76E-04	3.1099	0.032608		Creatinine	2	16
						PC aa C381	18	6
						PC ae C445	15	3
						PC ae C446	16	4
						SM C241	14	2
						SM C261	16	4
Significant metabolites identified by SAM						H1	16	4
Name	**d.value**	**stdev**	**rawp**	**q.value**		Orn	5	16
PC aa C420	-2.3571	0.057359	1.59E-04	0.013575		Tyr	4	15
Sarcosine	1.8523	0.39986	0.0019048	0.041695		PC aa C421	15	4
PC ae C425	-1.809	0.034421	0.0025397	0.041695		C141	17	7
PC aa C381	-1.7779	0.11341	0.0026984	0.041695		Sarcosine	6	16
SM C261	-1.752	0.068352	0.0030952	0.041695		Pro	7	16
SM C241	-1.7481	0.04232	0.0033333	0.041695		Cit	6	14
PC aa C386	-1.741	0.076451	0.0034127	0.041695		PC aa C425	14	6
Tyr	1.7058	0.071371	0.004127	0.04412		PC ae C406	14	6
PC ae C402	-1.6529	0.049348	0.0059524	0.047513		PC ae C381	17	10
Kynurenine	1.6416	0.077749	0.0063492	0.047513		PC aa C360	14	8
Orn	1.6367	0.080252	0.0064286	0.047513		PC aa C402	14	8
Creatinine	1.6314	0.044591	0.0066667	0.047513		lysoPC a C161	10	14
Significant metabolites identified by EBAM						lysoPC a C204	14	12
Name	**z.value**	**posterior**	**local.fdr**					
PC ae C425	-4.4025	0.991	0.0089989					
PC aa C420	-4.3851	0.99071	0.0092914					
SM C241	-3.7865	0.97241	0.027591					
Creatinine	3.4369	0.94737	0.05263					
PC ae C402	-3.3058	0.93646	0.063538					

Notes: For the fold change analysis the fold change threshold was set at 1.1 with a cut-off of 50% of pairs; the numbers represent the number of pairs with upregulated (count up) or downregulated (count down) values prior surgery. For SAM analysis the delta value was at 0.7 for an FDR value of 0.044; the q.value represent the FDR-adjusted p.value. For EBAM the delta value was set at 0.9 with an FDR of 0.032. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites

- PLS-DA results:

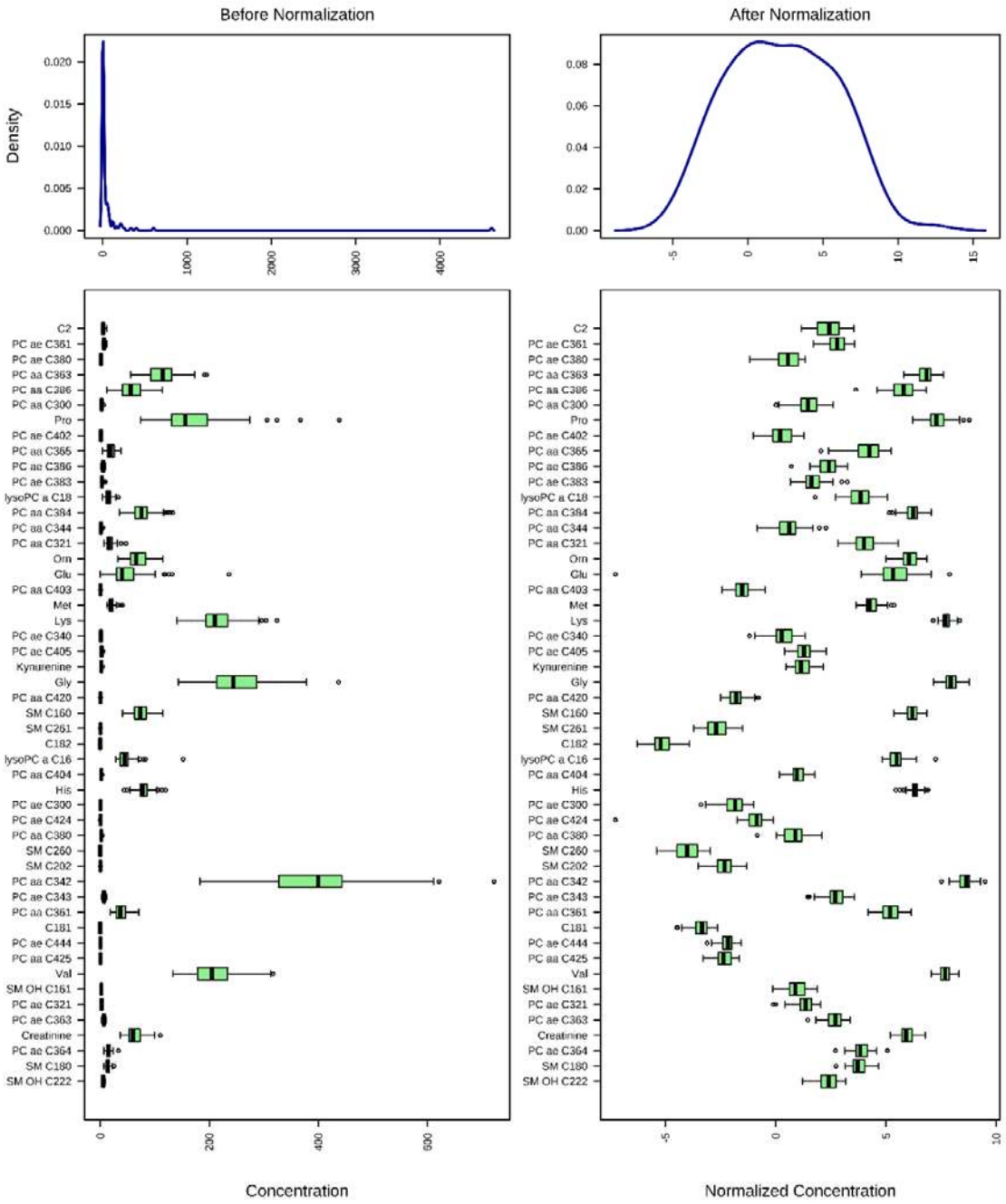
On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 1). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before ("0") and after ("1") surgery. In the right image, the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))



2.2 f) Statistical analysis for comparison before and after surgery in patients with preoperative BMI <25 and postoperative biochemical remission

- Normalization Result (60 samples, 30 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



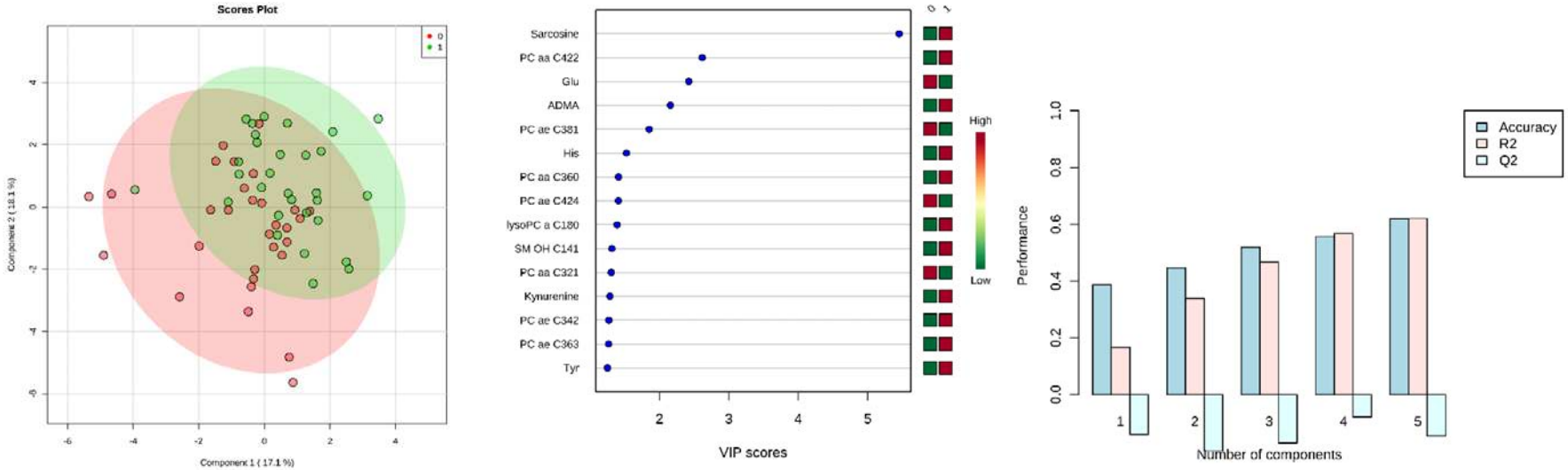
- Results of the performed statistical tests:

Significant metabolites identified by paired t-test analysis						***Significant metabolites identified by fold change***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Count (up)**	**Count (down)**
His	-4.2938	0.00017926	3.7465	0.022408		Tyr	4	16
						PC ae C445	15	3
						PC aa C321	15	6
						Leu	8	15
						ADMA	9	16
						Sarcosine	8	15
Significant metabolites identified by SAM								
Name	**d.value**	**stdev**	**rawp**	**q.value**				
His	1.7808	0.047587	0.00096	0.091361				
Significant metabolites identified by EBAM								
Name	**z.value**	**posterior**	**local.fdr**					
His	4.2938	0.94259	0.057412					

Notes: For the fold change analysis the fold change threshold was set at 1.15 with a cut-off of 50% of pairs; the numbers represent the number of pairs with upregulated (count up) or downregulated (count down) values prior surgery. For SAM analysis the delta value was at 0.5 for an FDR value of 0.046; the q.value represent the FDR-adjusted p.value. For EBAM the delta value was set at 0.9 with an FDR of 0.057. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites

- PLS-DA results:

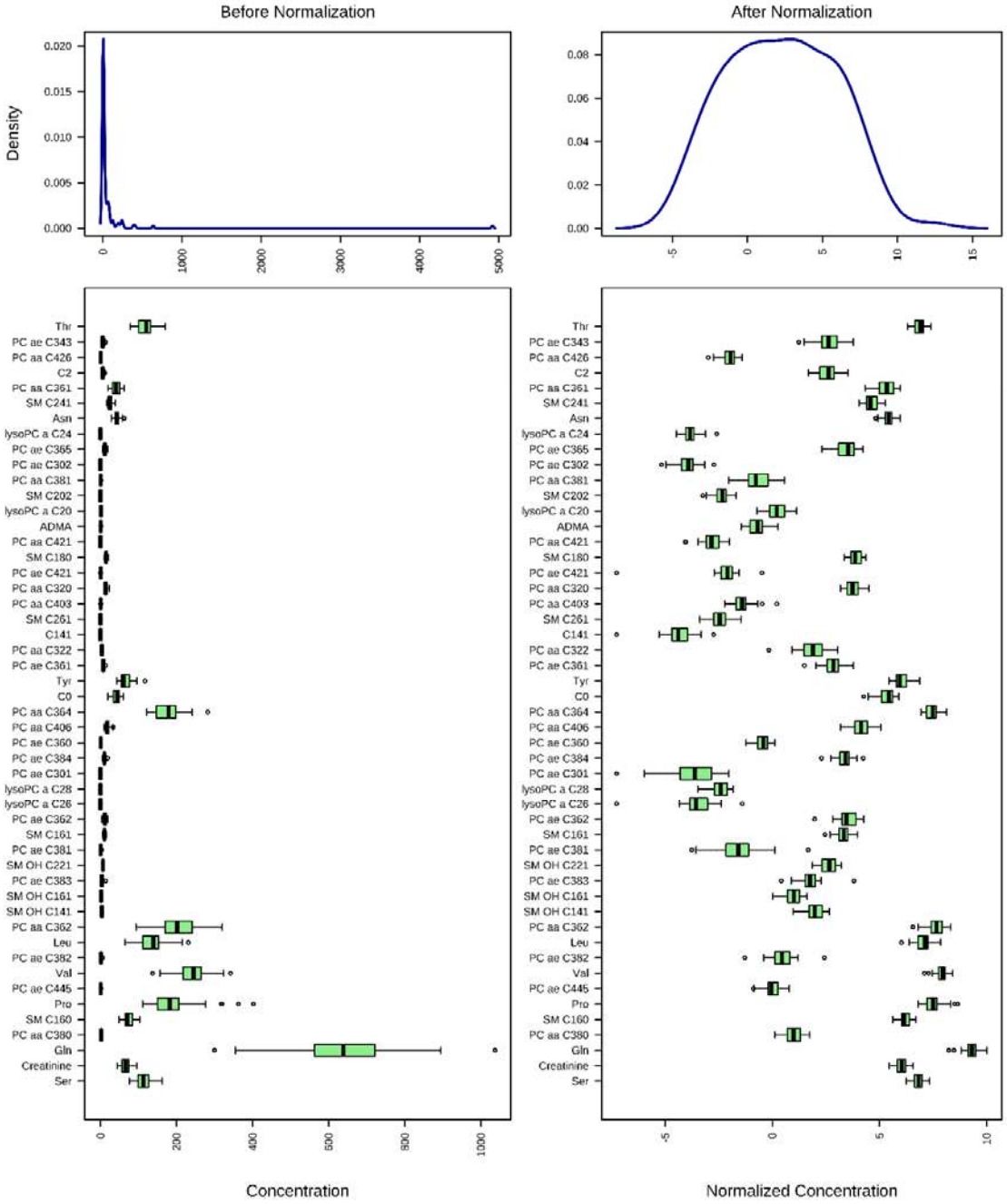
On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 1). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before ("0") and after ("1") surgery. In the right image, the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))



2.2 g) Statistical analysis for comparison before and after surgery in patients with preoperative BMI ≥25 and postoperative biochemical remission

- Normalization Result (40 samples, 20 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



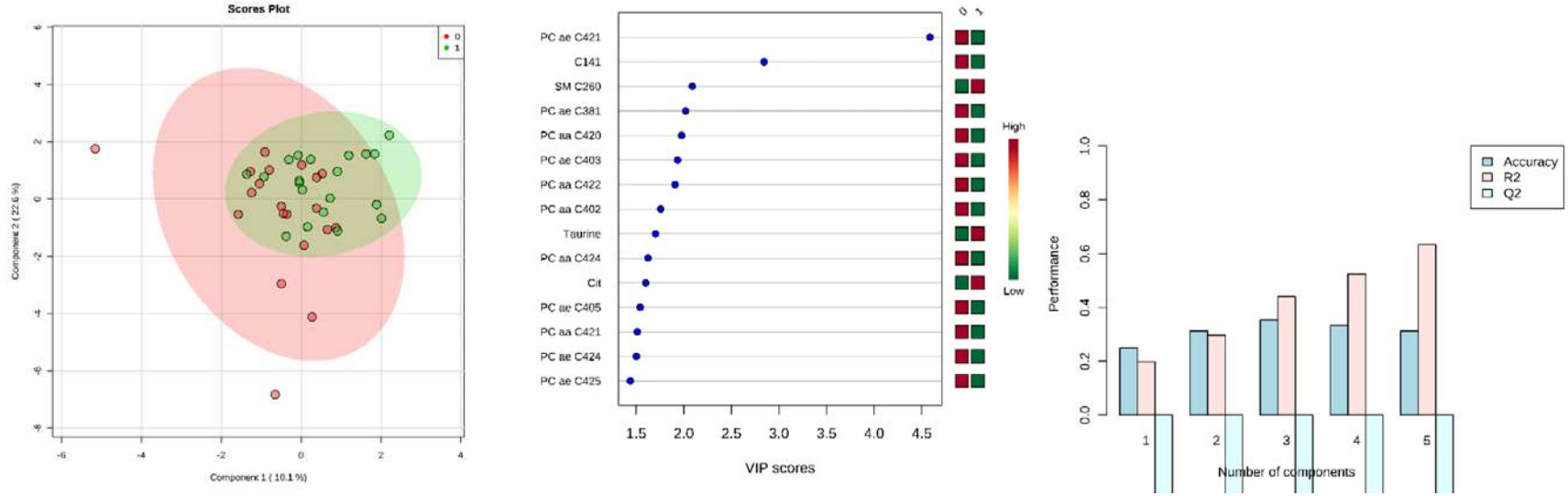
- Results of the performed statistical tests:

Metabolites (first 10) identifed by paired-t-test analysis (none resulted significant after correction for FDR)						***Significant metabolites identified by fold change***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Count (up)**	**Count (down)**
PC aa C420	2.618	0.016922	1.7715	0.89307		PC aa C386	11	3
PC ae C446	2.154	0.044285	1.3537	0.89307		PC aa C420	11	3
H1	2.1497	0.044668	1.35	0.89307		Glu	6	12
PC aa C361	-2.1262	0.046816	1.3296	0.89307		C141	10	5
C141	1.9486	0.066266	1.1787	0.89307		Orn	6	11
C0	-1.9361	0.067876	1.1683	0.89307		lysoPC a C180	5	10
PC aa C421	1.8636	0.077901	1.1085	0.89307		lysoPC a C170	7	10
PC aa C386	1.8332	0.082495	1.0836	0.89307		PC ae C381	10	7
SM C261	1.8148	0.085374	1.0687	0.89307		PC ae C301	8	10
Val	-1.7035	0.10478	0.97971	0.89307				
Metabolites (first 10) identified by SAM (none resulted significant after correction for FDR)								
PC aa C420	-2.618	0.091893	0.014524	0.92468				
PC ae C446	-2.154	0.057429	0.040238	0.92468				
H1	-2.1497	0.048494	0.040714	0.92468				
PC aa C361	2.1262	0.064917	0.042937	0.92468				
C141	-1.9486	0.17647	0.063016	0.92468				
C0	1.9361	0.084796	0.065	0.92468				
PC aa C421	-1.8636	0.095814	0.076111	0.92468				
PC aa C386	-1.8332	0.087574	0.080397	0.92468				
SM C261	-1.8148	0.095638	0.08373	0.92468				
Val	1.7035	0.059824	0.10302	0.92468				
Significant metabolites identified by EBAM								
none								

Notes: For the fold change analysis the fold change threshold was set at 1.15 with a cut-off of 50% of pairs; the numbers represent the number of pairs with upregulated (count up) or downregulated (count down) values prior surgery. For SAM analysis the delta value was at 0.22 for an FDR value of 0.783; the q.value represent the FDR-adjusted p.value. **Abbreviations:** FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites

- PLS-DA results:

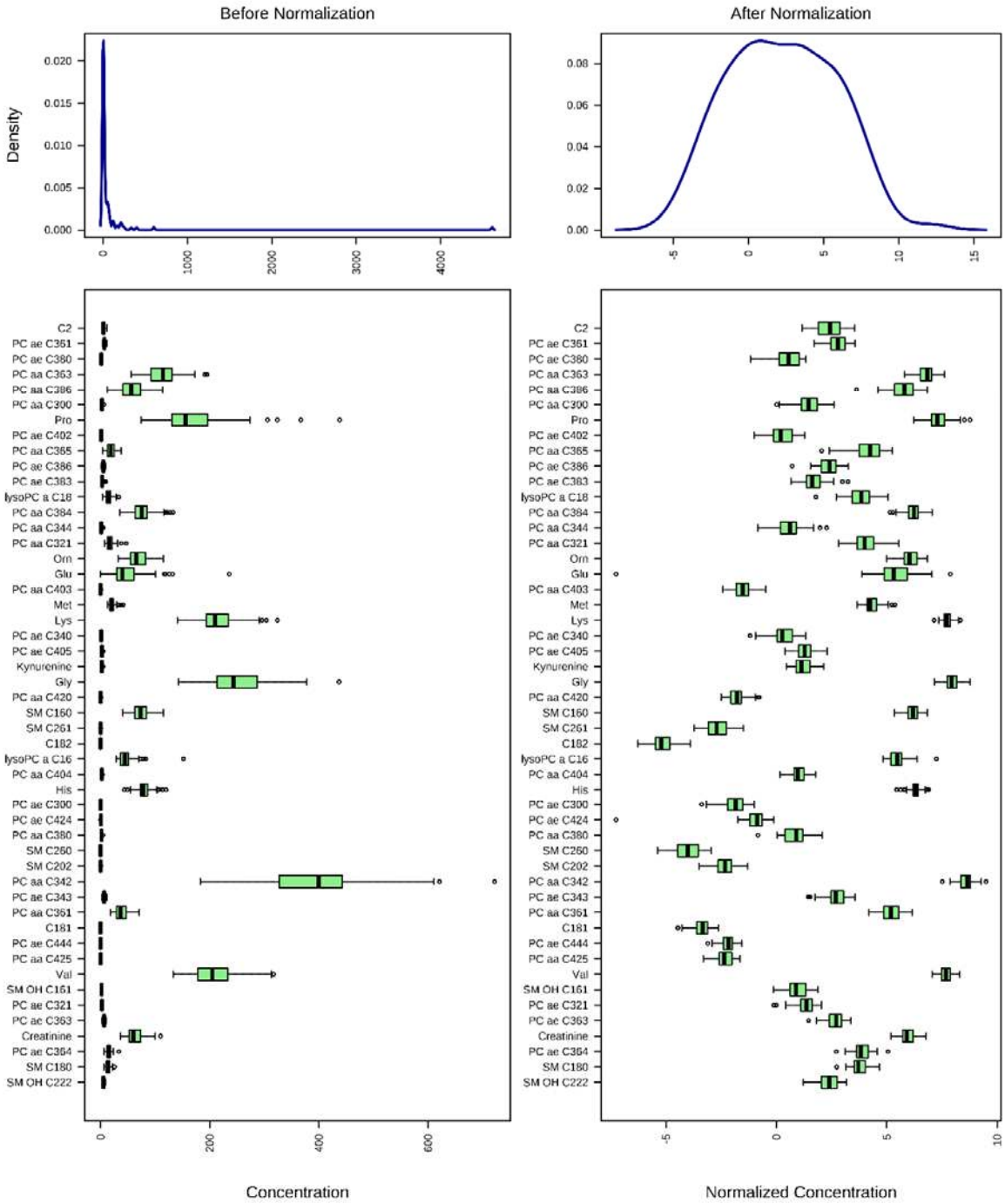
On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 2). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before ("0") and after ("1") surgery. In the right image, the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))



2.2 h) Statistical analysis for comparison before and after surgery in patients with preoperative age <45 and postoperative biochemical remission

- Normalization Result (40 samples, 20 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



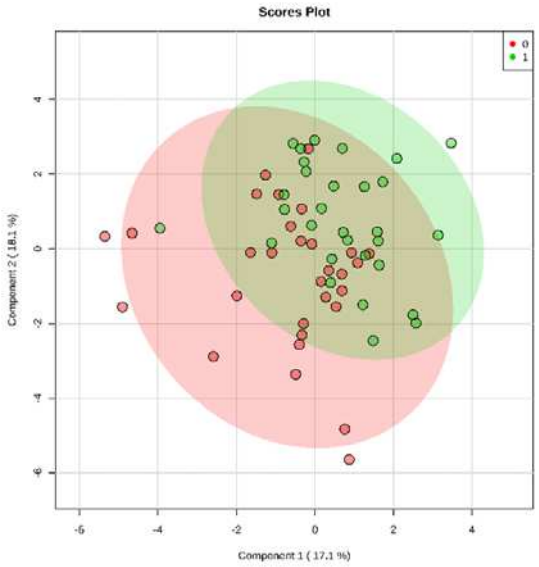
- Results of the performed statistical tests:

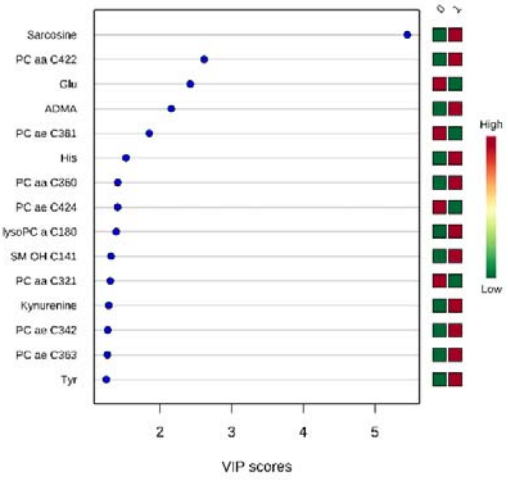
Metabolites (first 10) identifed by t-test analysis (none resulted significant after correction for FDR)						***Metabolites identified by fold change analysis (diabetes mellitus no VS yes)***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Count (up)**	**Count (down)**
PC aa C420	3.5511	0.0021328	2.671	0.18092		Glu	5	11
PC ae C445	3.2687	0.00404	2.3936	0.18092		ADMA	5	10
PC ae C425	3.2402	0.0043076	2.3658	0.18092		PC ae C381	10	5
PC ae C321	3.0709	0.0062903	2.2013	0.19815		PC ae C301	10	8
PC aa C421	2.8747	0.0097025	2.0131	0.22914				
His	-2.782	0.011879	1.9252	0.22914				
PC ae C446	2.7502	0.01273	1.8952	0.22914				
Orn	-2.4941	0.022013	1.6573	0.3467				
PC aa C386	2.3185	0.031723	1.4986	0.37574				
SM C241	2.2666	0.035284	1.4524	0.37574				
Significant metabolites identified by EBAM								
Name	**z.value**	**posterior**	**local.fdr**					
none								
Metabolites (first 10) identified by SAM (not significant after correction for FDR)								
Name	**d.value**	**stdev**	**rawp**	**q.value**				
PC aa C420	-1.2987	0.064439	0.007619	0.271				
PC ae C445	-1.1668	0.062044	0.014603	0.271				
PC ae C321	-1.1004	0.062415	0.019762	0.271				
PC aa C421	-1.0515	0.064461	0.024603	0.271				
PC ae C446	-1.0152	0.065396	0.029206	0.271				
PC ae C425	-	0.04716	0.038333	0.27836				
PC aa C386	-	0.078057	0.04	0.27836				
SM C202	-	0.10148	0.065714	0.32078				
PC aa C360	-	0.39268	0.067381	0.32078				
PC aa C320	-	0.068482	0.069206	0.32078				

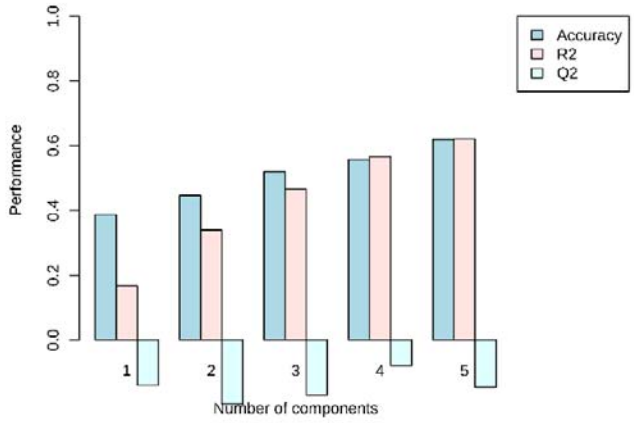
For the fold change analysis the fold change threshold was set at 1.2 with a cut-off of 50% of pairs. For SAM analysis the delta value was at 0.26 for an FDR value of 0.235; the q.value represent the FDR-adjusted p.value. For EBAM the delta value was set at 0.9 with an FDR of 0.
Abbreviations: FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites.

- PLS-DA results:

On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 2). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before (“0”) and after (“1”) surgery. In the right image, the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))


Scores Plot
0
1
Component 2 (18.1 %)
Component 1 (17.1 %)

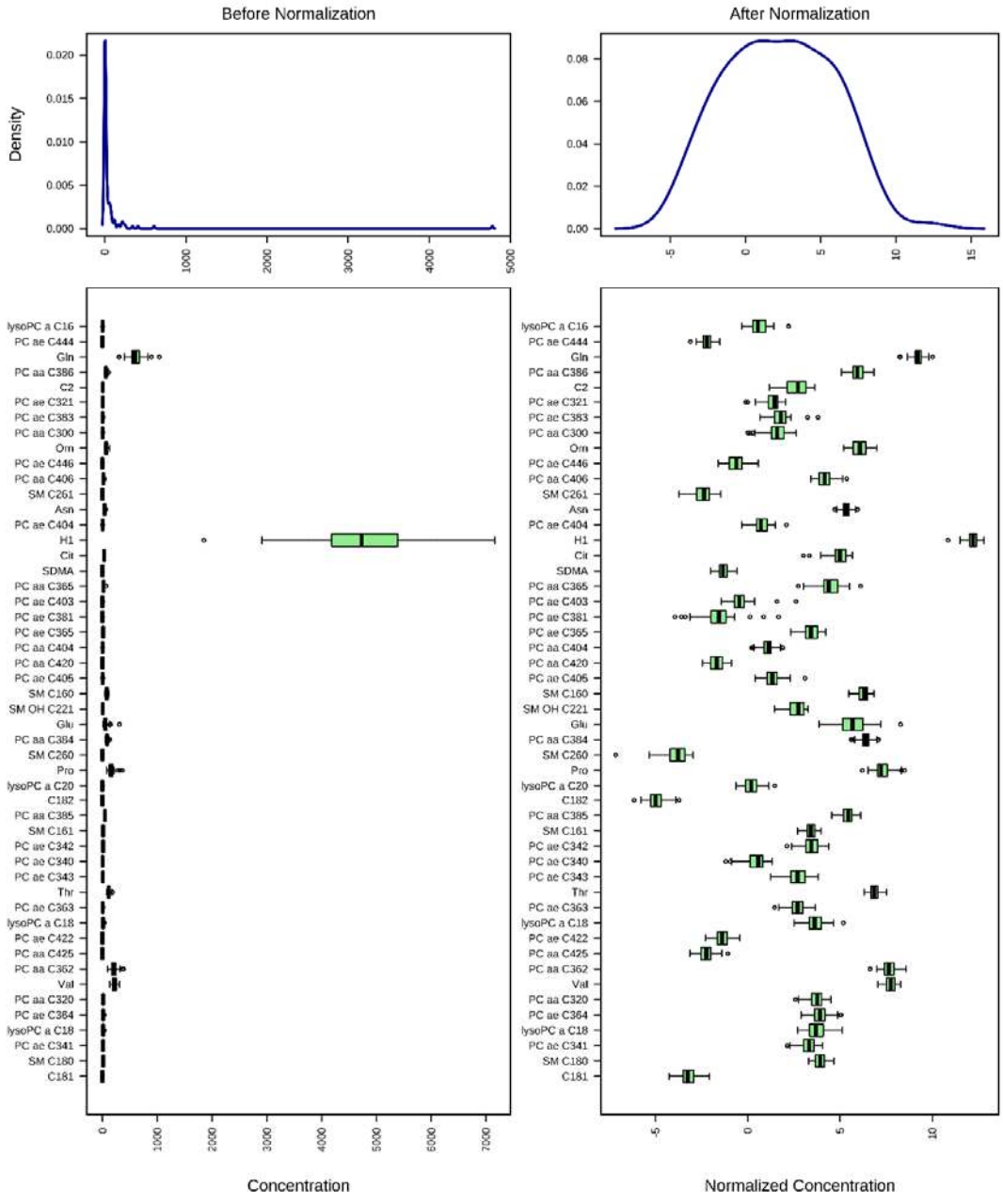

Sarcosine
PC aa C422
Glu
ADMA
PC ae C381
His
PC aa C360
PC ae C424
lysoPC a C180
SM OH C141
PC aa C321
Kynurenine
PC ae C342
PC ae C363
Tyr
High
Low
VIP scores


Accuracy
R2
Q2
Performance
Number of components

2.2 i) Statistical analysis for comparison before and after surgery in patients with preoperative age ≥45 and postoperative biochemical remission

- Normalization Result (66 samples, 33 pairs):

The boxplots (below) show at most 50 features/samples due to space limitation; the density plots (above) are based on all data.



- Results of the performed statistical tests:

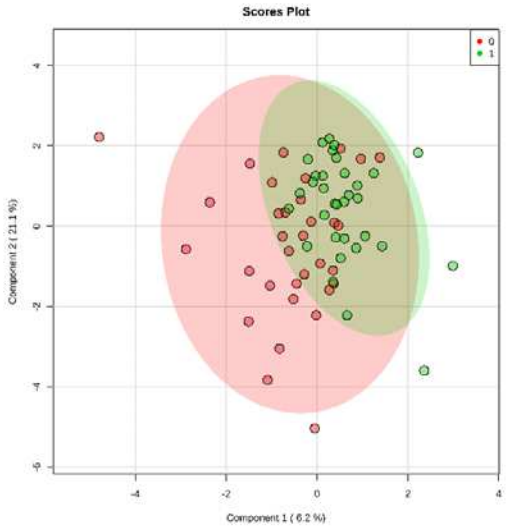
Metabolites (first 10) identifed by t-test analysis (none resulted significant after correction for FDR)						***Metabolites identified by fold change analysis (diabetes mellitus no VS yes)***		
Name	**t.stat**	**p.value**	**-log10(p)**	**FDR**		**Name**	**Count (up)**	**Count (down)**
Tyr	-3.6026	0.0010537	2.9773	0.089125		Tyr	2	16
PC ae C342	-3.3712	0.0019686	2.7059	0.089125		PC ae C342	3	17
SM OH C141	-3.3092	0.0023218	2.6342	0.089125		Kynurenine	3	16
H1	3.1972	0.0031189	2.506	0.089125		PC aa C420	16	5
PC aa C362	-3.0522	0.0045439	2.3426	0.089125		lysoPC a C180	6	16
Cit	-2.9877	0.0053599	2.2708	0.089125		C141	17	10
His	-2.9785	0.005487	2.2607	0.089125		PC aa C321	17	10
PC aa C281	-2.9695	0.0056142	2.2507	0.089125				
Kynurenine	-2.8501	0.0075858	2.12	0.094062				
PC ae C364	-2.8283	0.0080096	2.0964	0.094062				
Significant metabolites identified by EBAM								
Name	**z.value**	**posterior**	**local.fdr**					
Tyr	3.6026	0.96164	0.038362					
PC ae C342	3.3712	0.94808	0.051921					
SM OH C141	3.3092	0.9437	0.056297					
Metabolites (first 10) identified by SAM (not significant after correction for FDR)								
Name	**d.value**	**stdev**	**rawp**	**q.value**				
ADMA	0.78592	0.2905	0.016142	0.19155				
Tyr	0.72354	0.048712	0.02315	0.19155				
Kynurenine	0.71887	0.06538	0.023701	0.19155				
Taurine	0.71078	0.10656	0.024488	0.19155				
PC ae C342	0.69219	0.050081	0.02685	0.19155				
Cit	0.66066	0.055029	0.031969	0.19155				
His	0.66061	0.055243	0.031969	0.19155				
PC ae C364	0.63249	0.055833	0.036929	0.19155				
PC ae C363	0.61337	0.053844	0.041654	0.19155				
SM C260	0.60994	0.12675	0.042598	0.19155				

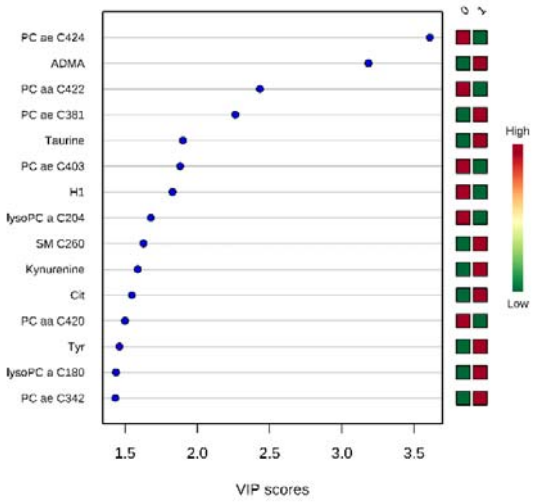
For the fold change analysis the fold change threshold was set at 1.15 with a cut-off of 50% of pairs. For SAM analysis the delta value was at 0.25 for an FDR value of 0.146; the q.value represent the FDR-adjusted p.value. For EBAM the delta value was set at 0.93 with an FDR of 0.049.
Abbreviations: FDR, false discovery rate; n.s., not significant; SAM, significance analysis of microarray/metabolites; EBAM, empirical bayesian analysis of microarray/metabolites.

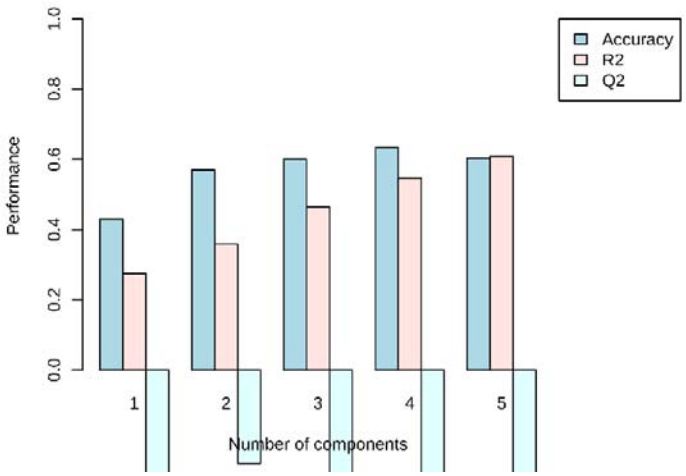
- PLS-DA results:

On the left the scores plot with 95% confidence interval are represented. For each component the percentage of explained variability is represented in the brackets. In the middle are represented the important features of the component with higher percentage of explained variability (in this case component 2). Colored boxes on the right indicate the relative concentrations of the corresponding metabolite before (“0”) and after (“1”) surgery. In the right image, the cross-validation results are represented. Q2 is an estimate of the predictive ability of the model, and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have low PRESS

or high Q2. **Negative Q2**, which means that the model is not at all predictive or is overfitted (images and commentary from the Metaboanalyst (1))







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1. Xia J, Wishart DS. Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis. Curr Protoc Bioinformatics 2016; 55:14 10 11-14 10 91